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GASTRIC RESPONSE TO FOODS[1]

X. The Psychic Secretion of Gastric Juice in Normal Men

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As early as 1852 it was shown by Bidder and Schmidt (1) that the mere sight of food called forth the secretion of gastric juice in the dog. It remained, however, for Pavlov (2), some forty years later, to establish more definitely the character of the so-called "appetite" or "psychic" secretion of the gastric juice. He pointed out that such a secretion was the normal initiator of gastric digestion in dogs, and might be induced by the sight, smell, taste, mastication or thought of food, or even through the stimulation of appetite by the presence of solid matter in the stomach. This was further emphasized by the fact that very appetizing foods, such as meats, induced a greater secretion in "sham feeding" experiments than milk or bread, which were not so greatly relished by the animals.

The results of experiments on man have been by no means so conclusive. Thus Carlson (3) was able to point out probable sources of error in most of the earlier investigations. This author concluded from the work of Homborg (4) and his own experiments that in spite of apparent evidence in the literature to the contrary, what Pavlov found true for animals was also true for man, namely, that gastric secretion was not induced by the chewing of indifferent substances nor by the taste or smell of chemical substances not arousing the appetite sensation.

[1] The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

Accepting the view that the initiation of gastric secretion is dependent upon arousing or augmenting the appetite sensation, it becomes of immediate interest to determine whether the thought, sight, smell or taste of food exerts the greatest influence in this direction as well as to estimate the absolute importance of the appetite secretion as a factor in gastric digestion.

Carlson found that no secretion could be induced in his gastric fistula subject by the thought of food, and that the secretion produced by seeing or smelling food was relatively slight and inconstant, the significant appetite secretion being that induced by the tasting and chewing of good food. Luckhardt (5), on the contrary, employing a Rehfuss stomach tube and using as a subject a completely normal man, found under good experimental conditions that the combined sight and smell of food markedly increased the flow of gastric juice.

Pavlov believed the appetite secretion to be of very great importance in initiating gastric digestion. This is discounted by Carlson, who found in his human subject, as well as in cats and dogs, that the continuous secretion of the stomach served a similar purpose, and that elimination of the appetite secretion did not cause indigestion.

That the unpalatable condition of a food need not necessarily influence its ultimate digestion and utilization in the alimentary tracts of normal men was indicated by work carried out in the laboratory of Atwater, who found that nauseating meat, the so-called "embalmed beef" fed to soldiers in the Spanish-American war, was still well utilized. That the economy of man is well served under such circumstances must remain doubtful.

In this connection it is also necessary to consider psychic or other influences tending to inhibit the development of the appetite secretion; bearing in mind that emotional excitement may destroy the motor (6) as well as the secretory (7) activities of the stomach.

The present paper is a contribution toward determining the relative importance of the various factors involved in the appetite stimulation of gastric secretion, as well as toward estimating the influence of the appetizing or unappetizing character of a meal and of the mental attitude of the subject upon the gastric response and the ultimate digestion of food.

Following the appetite stimulation in each case, the stomach was emptied at regular intervals, using a Rehfuss stomach tube, the volume of secretion determined, its free and total acidity, pepsin and amino-acid nitrogen estimated according to procedures previously described (8).

The sight psychic secretion and the effect of smell. Experiments were carried out to determine whether the sight of food alone, or the sight and odor of food combined gave rise to the production of any gastric secretion, as well as to compare the effects on such secretion of the sight of foods prepared in an unpleasing manner.

A breakfast table was set, in a small well-lighted laboratory, with clean linen, bright chinaware, and the following foods were served in a pleasant manner and at one time: ham and eggs, oranges, shredded wheat, bread and butter, hot coffee, cream and sugar. Subjects were brought to this room after any residua had been removed and the level of continuous secretion established. The nose was kept lightly but effectively clamped throughout.

In figure 1 are charted the results obtained with a subject who was a laboratory helper, accustomed to eating lunch at the laboratory. Total volumes of secretion removed are charted with total and free acidities, amino-acid nitrogen values and a so-called total actual acidity which indicates the product of the volume by the total acidity and represents the total amount of acid in the gastric samples.

It will be noted that this subject showed a marked response to the sight of food as regards the volume of gastric juice secreted and the "total actual" acidity. The continuous secretion in this case was high and a greater augmentation by psychic stimulation might perhaps be expected. Under similar conditions another subject (fig. 2) showed a lower and less acid continued secretion. Sight augmented the volume but slightly, although the acidity was distinctly increased.

Somewhat less accustomed to eating in the laboratory were the subjects of the experiments charted in figures 3, 4 and 5, which were carried out in exactly the same way except that clamps were not used. The distinct but not voluminous secretion in these cases may be attributed mainly to the effect of sight as the odor of the meal was not pronounced and odor was found to have little influence on psychic secretion in these subjects. The subjects were, of course, led to believe they would actually receive the food.

To determine whether any elaboration in setting was requisite for the stimulation of secretion by sight, the meal was simplified to a simple half grape fruit served in the usual way. The odor being imperceptible, the noses were not clamped. The results are charted in figures 6 and 7. A stimulation of secretion was brought about by the sight of the food in both cases. It is also probable that some of the psychic secretion may leave the stomach during the intervals of the experiment with the

increase in gastric tonus and produce a stimulation of the pancreatic secretion.

The effect of allowing subjects to seat themselves at a breakfast table prepared in an unpleasing manner was also tried out on six subjects. The same table was used as in the preceding experiments, and the same foods were served. However, the ham and eggs were scorched; the shredded wheat biscuits and bread roughly broken; the coffee and milk weak and diluted; the butter soft; sugar lumpy and dark; the oranges partly squeezed; the dishes generally somewhat greasy and with an appearance of dirtiness induced by the use of charcoal. Newspapers were used in place of linen. The noses of the first two subjects were clamped, of the others free.

The results of these experiments are charted in figures 8, 9, 10, 11, 12 and 13. In only one of these cases (see fig. 12) was any secretion induced above the level of the continuous secretion. It is evident, therefore, that food served in an unpleasant manner will not give rise to an appetite secretion under ordinary conditions, although custom and degree of hunger will naturally influence the conception of an appetizing food.

Breakfasts served in a pleasant manner and with appetizing foods were set before each of the six subjects just mentioned from 15 to 30 minutes after they had been presented with a view of a breakfast of the opposite and discouraging character and which had evoked no psychic gastric response. The results are plotted in the same charts as the preceding tests (figs. 8, 9, 10, 11, 12 and 13).

It will be noted that in the first two cases a marked appetite secretion followed the presentation of the second meal, this being a sight effect as noses of these subjects were clamped. However, the other four subjects did not show a psychic secretion under these conditions. The subjects showing a response were accustomed to eating in the laboratory and may have felt that they would not be expected to actually partake of the disagreeable food. The other subjects having no knowledge of the character of the test might well be more strongly repulsed by the first meal, this effect being carried over for the period of 15 to 30 minutes until the palatable meal was set before them. A secondary effect might also be their suspicion that since they were not permitted to partake of the first meal they might not have a chance at the second, although the contrary view was impressed upon them.

The psychic secretion and the odor of food. The influence of the odor of food alone on psychic secretion was tried out on seven subjects (see

figs. 14 to 20). The odor of frying beefsteak was used as a stimulus, the odor being pleasant, strong and unmistakably that of an appetizing food of common consumption. Subjects were blindfolded and the ears were muffled in order to exclude the influence of the sight of the steak and of hearing it fried. Subjects inhaled liberally the fumes arising from the frying steak. As in our other tests, the subjects had had no food for fourteen hours.

Three of the subjects showed no increase in the volume of secretion under the influence of these odors. The four others showed some increase, but in no case was the secretion voluminous. It would appear from these tests that odor was considerably less important than sight in inducing the appetite secretion, at least in man. These results are supported by the experiments previously mentioned, in which combined sight and odor of food brought forth no greater secretion than sight alone. Odor may have an influence on the motor activity of the stomach and may be of importance in animals with a more highly developed sense of smell. There may very well also be considerable differences in individuals of the human species.

The psychic secretion and the tasting and chewing of food. The subjects of the preceding tests on the influence of odor were permitted to rest for half an hour to reëstablish the level of continued secretion which had in most cases been little affected. They were then permitted to chew for five minutes portions of tenderloin steak with strict caution to swallow none of the pieces, this possibility being checked by careful examinations of the gastric contents. In all cases the subjects remained blindfolded. In the first four cases the noses of the subjects were also clamped so that none of the vapors could be inhaled by that channel. The results are charted in figures 14, 15, 16 and 17, and show no distinct influence of tasting and chewing meat under these conditions upon the secretion of gastric juice. In one case the volume of gastric contents was somewhat increased, but the low acidity shows that very little acid gastric juice could have been secreted. Apparently the taste and chewing of food in the absence of sight or odor produced no marked psychic secretion.

In three other cases the same procedure of chewing and tasting beefsteak was carried out, but the noses of the subjects were unclamped, sight, however, being excluded (see figs. 18, 19 and 20). As illustrated in these cases the influence of the combined tasting, chewing and smelling of food on the secretion of appetite gastric juice was very pronounced and was much greater than that of smell alone.

The influence of the sound or thought of food. Subjects were blindfolded and had their noses clamped to exclude the sight and smell of food. They were seated before a frying pan in which a steak was being broiled with plainly audible sputtering and sizzling. They were told what a fine, juicy steak was being prepared for them and a general attempt made to keep their attention on the subject of appetizing meats. The results are charted in figures 21 and 22. In one case the result was negative; in the other case a distinct stimulation of secretion resulted. The variation must be ascribed to individual differences.

After one-half to three-quarters of an hour rest, the nose clips were removed and the subjects permitted to smell as well as hear the sputtering of frying steak. Results are plotted on the same charts and show that in one case a very slight rise in secretion took place. In the other case a definite stimulation occurred, although the earlier level for hearing and thought of food was not surpassed. In one case the subject was permitted to smell feces of a repulsive odor fifteen minutes after smelling steak. Any psychic secretion appears to have been depressed to the level of the continuous secretion but not below this.

Experiment 23 gives a comparsion of the psychic effects of: *a*, the sight of a frying steak (ears not stoppered); *b*, sight and smell; and *c*, taste of the same food. A distinct stimulation was produced by the sight of the food. One-half hour later the sight and odor of similar food produced a very similar stimulation. After a further interval of 15 minutes the taste of the food gave a lesser stimulation than sight or sight and smell had previously done.

A summary of some of the results obtained on two of our subjects in so far as volumes and "total actual acidities" of appetite secretions were concerned, is given in figures 24 and 25. They must, of course, be considered in connection with details of individual experiments. They serve, however, to emphasize the important rôle of the sight of food as a stimulus to the appetite.

The influence of palatability or unpalatability of a meal on its gastric digestion. Two subjects were given uniform meals prepared and served in the ordinary manner. On a later day they were given the same foods prepared in as unpalatable a manner as possible without altering their chemical composition. The meal used consisted of: cream of wheat, 100 gm.; sugar, 10 gm.; milk, 35 cc.; coffee, 100 cc.; graham crackers, 50 gm.; oranges, 50 gm.; water, 100 cc. On the second experimental day these foods were all mixed together in a conglomerate

mass, discolored with small amounts of burnt crackers and charcoal, and the atmosphere at the table saturated with the repulsive odors of valeric and butyric acids.

The first of these subjects was of a nervous temperament and from his statements and manner was judged to be easily influenced or disturbed by the character and preparation of his food. In fact he positively refused on one occasion to continue eating a meal of the second type mentioned above, although urged to do so in the interest of science. The results on this subject (see figs. 26 and 27) show no delay or inhibition of the acid response of the stomach, although the evacuation time was somewhat prolonged.

The second subject was accustomed to eating in a laboratory, was of a phlegmatic temperament, claiming and appearing to be very little disturbed by the appearance of food or the condition in which it was served. The results on this subject are given in figures 28 and 29. The unpalatable food showed a rapid, though not quite so rapid, development of acidity and a few minutes quicker evacuation.

The first subject was also tried out with a palatable meal 50 minutes after he had violently refused one which he believed to be contaminated. The result is charted in figure 30. The development of acidity was even more rapid than in the case where the meal was given under normal conditions. If any depression of psychic secretion was carried over through this interval, there were no signs of it.

The first subject was also given a meal of unpalatable character similar to the ones already described, but prepared by himself and hence known by him to be innocuous. The results as charted in figure 31 show a rapid development of acidity and quick evacuation.

Some information with regard to the gastric response to foods unpalatable in appearance, odor and taste was obtained by experiments on the feeding of Chinese preserved duck eggs called "pidan." These eggs have dark greenish yolks and yellow-brown "whites" of a firm, gelatinous consistency and possess distinct odors of ammonia and hydrogen sulphide. One subject disliked these eggs but did not know what they were and was not especially prejudiced against them. The other subject, "Don," was of a nervous type, and just as he finished eating the eggs he was told in a joking manner by one of the laboratory wits that they were of prehistoric Chinese origin. The subject became clearly suspicious that something had been given him that was not entirely fresh. The results of this test as compared with similar tests on boiled duck eggs and on raw white and yolk of egg are given in

figure 32. They show a depression of gastric secretion after "pidan" lasting for an hour and a quarter, the acidity then rising rapidly to normal figures. This delay may have been due to inhibition of appetite secretion, gastric activity being finally aroused through chemical stimulation following the solvent action of the slow continued secretion.

The failure of this unappetizing food to arouse the secretory or motor activities of the stomach to a normal response is indicated also by our results on the first subject mentioned above. The curves are given in figure 33 and show that while raw hens' eggs gave an acid response of over 100 in the first hour and left in 2¼ hours, the preserved eggs at no time gave acidities of over 30 and remained in the stomach 4½ hours. It appears that the unappetizing character of these eggs led to a delayed acid response and slow evacuation, perhaps complicated by their failure to show some early digestion with consequent chemical stimulation.

Influence of prejudice against a food on its digestibility in the stomach. It is very common to find people who have a prejudice against certain foods generally classed among the most wholesome articles of diet. Certain cases may be due to a food anaphylaxis or sensitivity. Others may be due to defective gastric or intestinal digestion or other causes. Undoubtedly many have no foundation and are the results of wrongly placing the blame of certain digestive disturbances.

One of our subjects, "Ham," had a strong prejudice against eggs in any form and had not eaten them for years. He was with difficulty persuaded to take eggs prepared in several different ways. The results of these tests are plotted in figure 34, and show that eggs were digested by this subject in a perfectly normal manner, at least as far as the stomach was concerned. Neither did untoward symptoms of any kind develop.

Influence of newspaper reading on gastric digestion. Subjects were permitted to read newspapers throughout the course of a meal of palatable foods, the same test meal as used in previous experiments. The gastric responses of two subjects who read newspapers and responses of same subjects with no reading but with usual table talk are charted in figures 26, 28, 35 and 36. No distinct influence of newspaper reading was noted. Responses were quite normal in all cases. The slight differences in acid development and evacuation time were in one case favorable and in the other case unfavorable to newspaper reading.

Influence of the unpalatable character of a diet on its ultimate utilization by the human body. Smith, Holder and Hawk found (9) in a metabolism experiment on a normal man that where a uniform diet of a

palatable character was given for several days, followed by a period in which the same foods were jumbled together in dirty dishes and served amidst ill-smelling and otherwise unpleasant surroundings, that the nitrogen utilization in the first case was 86.7 per cent and in the second, 85.6 per cent. The nitrogen balance showed a retention in the first period of 3.0 per cent and in the second, of 6.4 per cent. This in spite of the fact that the subject was only with difficulty persuaded to eat the unpalatable food and that another subject who was given the same kind of food became nauseated and could not continue.

Influence of anxiety on gastric digestion (10). The study of the influence of emotional strain on digestion in man offers some difficulties due to the fact that the emotions cannot be readily controlled, nor are the subjects of extreme emotion readily amenable to experimentation. We were, however, able to obtain an interesting illustration of the profound effect of mental anxiety on gastric digestion in the case of one of our subjects. The man was a first-year medical student who had previously served as a subject of gastric tests. He was given 100 grams of fried chicken on the morning of an important examination in chemistry and was asked to write out his answers during the course of the test. He was plainly worried over the outcome of the examination and of his year's work. The effect upon gastric digestion was the prolonging of the evacuation time to $6\frac{1}{4}$ hours. The intra-gastric acidity remained in the neighborhood of 90 for 3 hours. The normal digestion curve for fried chicken on this subject was obtained a week later under the best mental conditions. The time required was $4\frac{1}{4}$ hours and the maximum acidity about 65. It is not at all surprising that worry aggravates a condition of gastric ulcer.

An interesting experiment on the digestion of milk in the human stomach may be cited in this connection (11). It was found that in the stomach of one of our subjects milk would not curdle. The test was carried out at the end of the year immediately before the final examinations. The subject was one of the most brilliant students in his class and had worked hard. We made several tests on this student and in every case milk left his stomach rapidly and without curdling. He digested all other food normally. The next fall, upon his return to college, we made another milk test upon him and found that his stomach curdled milk in a normal manner. At this time he was in fine physical condition, having had a long, pleasant vacation, whereas in the spring he was in a highly nervous state as a result of his hard study. This serves to illustrate the influence which rigid and prolonged mental application may exert upon the stomach in certain types of individuals.

SUMMARY AND CONCLUSIONS

The sight alone of a table well set with nourishing foods was found to give rise to a distinct secretion of gastric juice in normal men. The sight of a half grape fruit only resulted likewise in an appetite secretion. The sight of the same foods illy prepared and poorly served resulted in no stimulation of appetite secretion. The service of a well prepared meal half an hour after the service of a poorly prepared one gave in some instances a distinct secretion, in others not.

The odor alone of frying meat produced in some cases no appetite secretion, in others a slight secretion. Odor alone produced less stimulation than sight alone.

The tasting and chewing of food in the absence of smell or sight produced no marked psychic secretion. The combined influence of the tasting, chewing and smelling of food was pronounced and much greater than that of smell alone.

The sound and thought alone of a frying steak gave rise to a gastric secretion. The influence of smell with hearing produced little additional effect. Evil odors depressed secretion to the level of the continuous secretion.

In consecutive tests the sight of food, with and without odor, produced similar degrees of stimulation, while taste alone had less effect.

Mixed meals consisting of nourishing ingredients but very unpleasantly prepared and served gave rise in the case of a phlegmatic individual to no distinct delay in the development of intra-gastric acidity or in evacuation. A more susceptible individual showed a slight delay in evacuation time, but none in acid response.

Chinese preserved eggs, unpalatable to our subjects in appearance, odor, taste and belief in their unwholesome character led to delayed acid response and evacuation. In one case the normal acid level was ultimately attained due to chemical stimulation.

In one subject a strong prejudice against eggs was found not to result in any abnormal gastric response when eggs were eaten.

The ultimate utilization of the protein of a diet prepared in a most unpalatable manner was not found to be appreciably less than that of the same diet served under the best conditions.

Newspaper reading during the course of a meal could not be shown to have any distinct influence on gastric digestion.

Anxiety and mental strain were found to markedly delay gastric digestion.

The authors desire to thank for their coöperation the students of Jefferson Medical College who acted as subjects of these tests.

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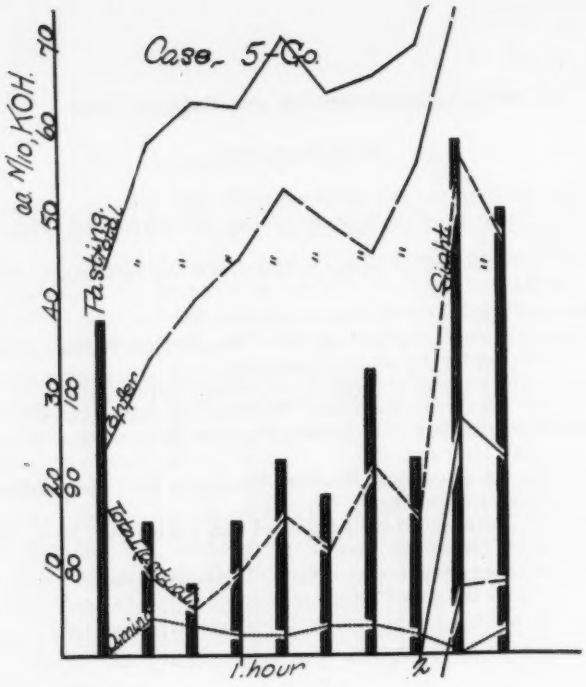
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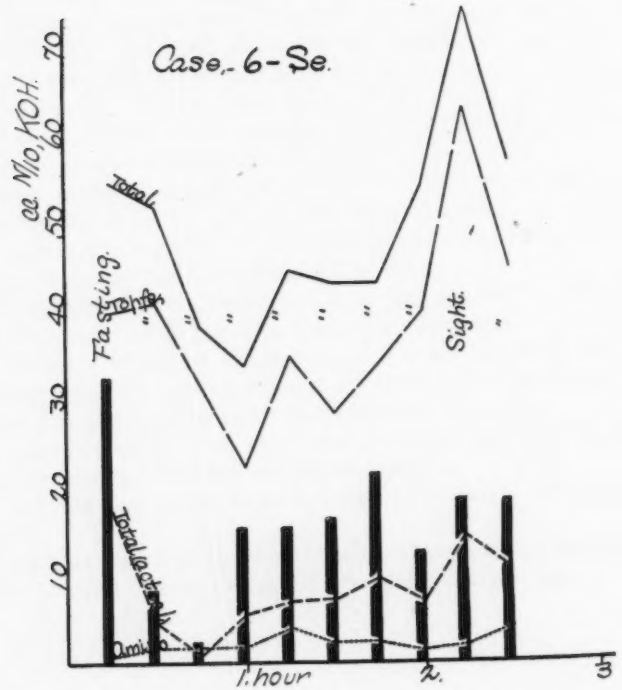
ca N/10 KOH.
Fasting.
Sight.
1. hour
2.

FIG. 1.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Fasting.
Sight.
1. hour
2.
3

FIG. 2.

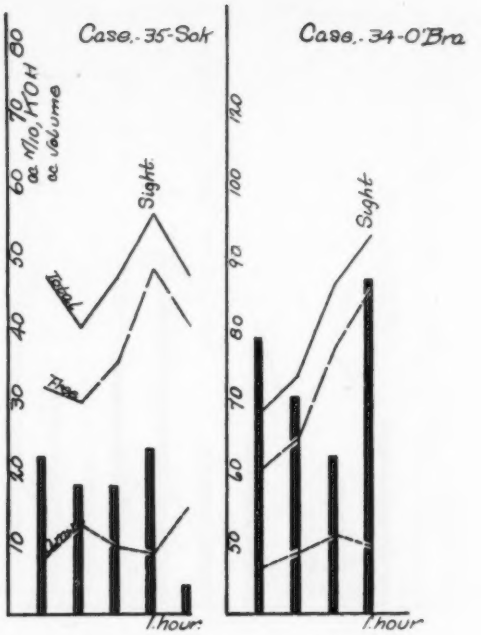


FIG. 3. FIG. 4.

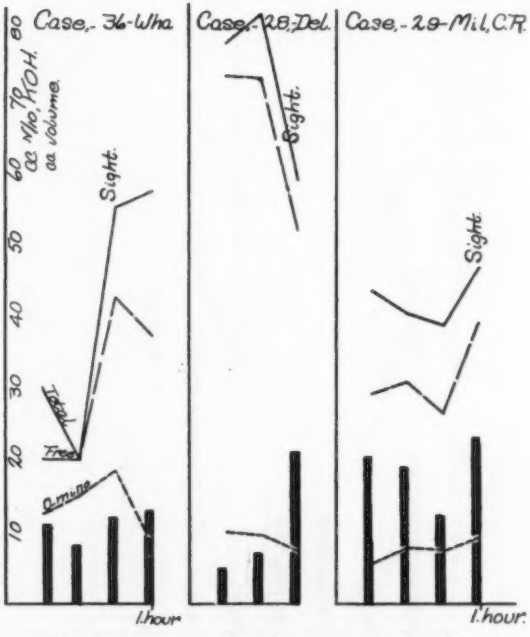


FIG. 5. FIG. 6. FIG. 7.

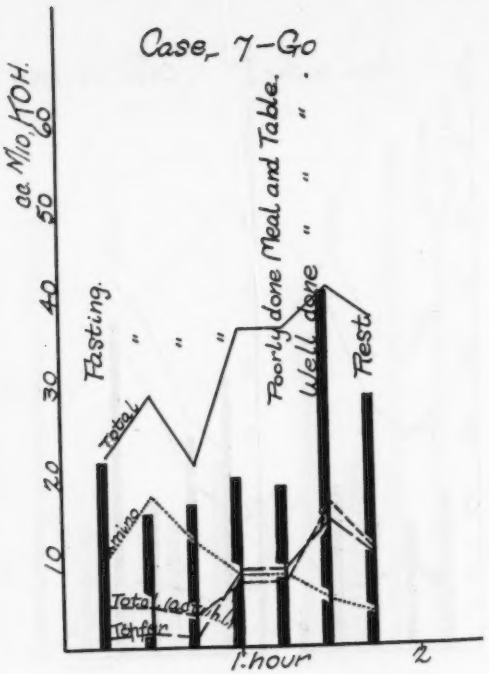
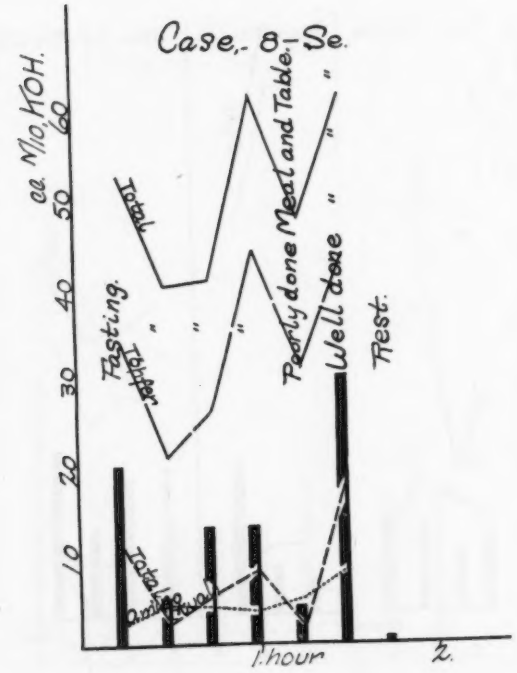

Case, 7-Go
cc. N/10 KOH.
10
20
30
40
50
60
Fasting.
" "
Poorly done Meal and Table.
Well done " " "
Rest.
Total
Amino
Total Lactic Acid
Tchfor
1. hour
2

FIG. 8.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cc. N/10 KOH.
10
20
30
40
50
60
Fasting.
" "
Tchfor
Total
Poorly done Meal and Table.
Well done " "
Rest.
Total Lactic Acid
Amino
1. hour
2.

FIG. 9.

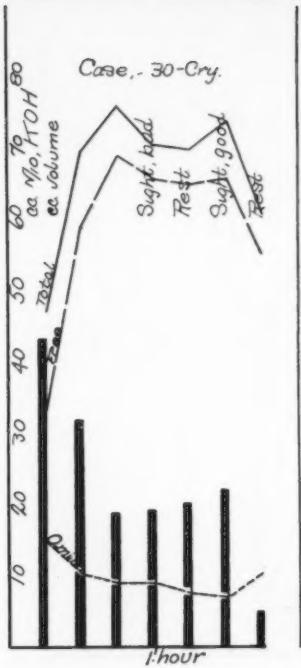

FIG. 10.

FIG. 11.

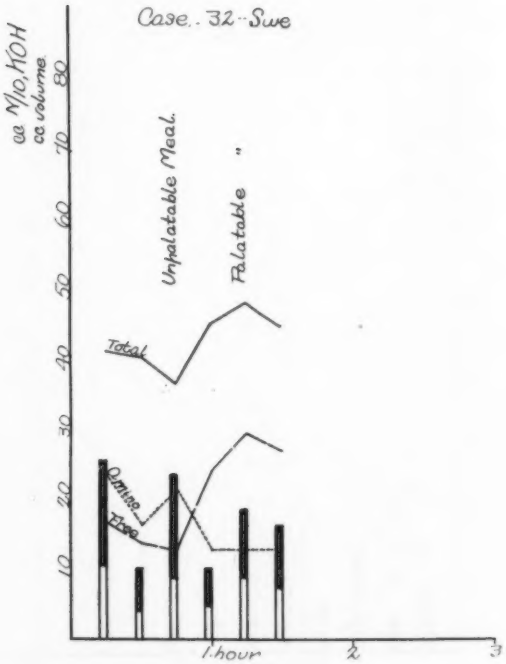

FIG. 12.

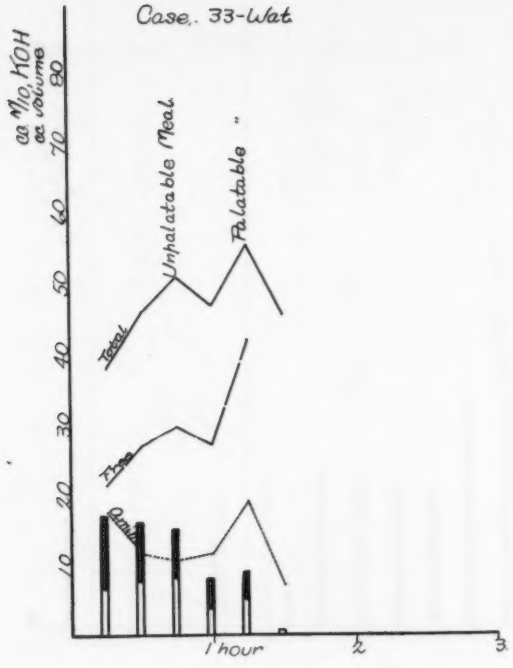
Case. 33-Wat
cc N/10 KOH
cc Volume
10
20
30
40
50
60
70
80
Unpalatable Meal
Palatable "
Total
Free
Amino
1 hour
2
3

FIG. 13.

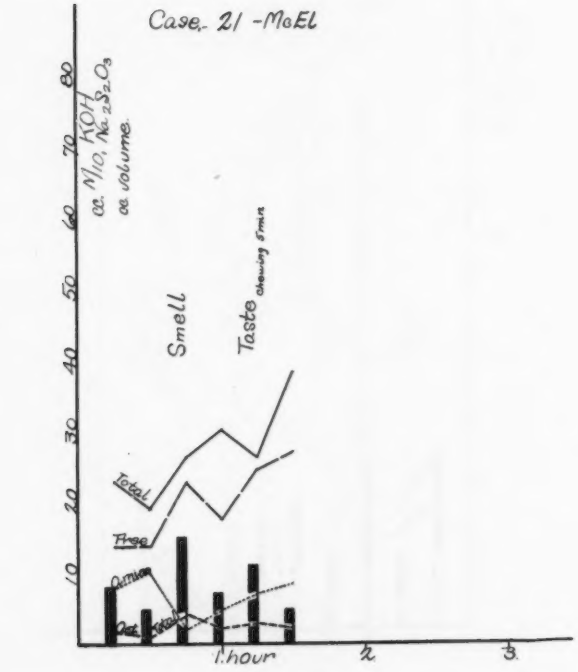
Case. 21 -McEl
cc N/10 KOH
cc Volume
10
20
30
40
50
60
70
80
Smell
Taste
Total
Free
Amino
1 hour
2
3

FIG. 14.

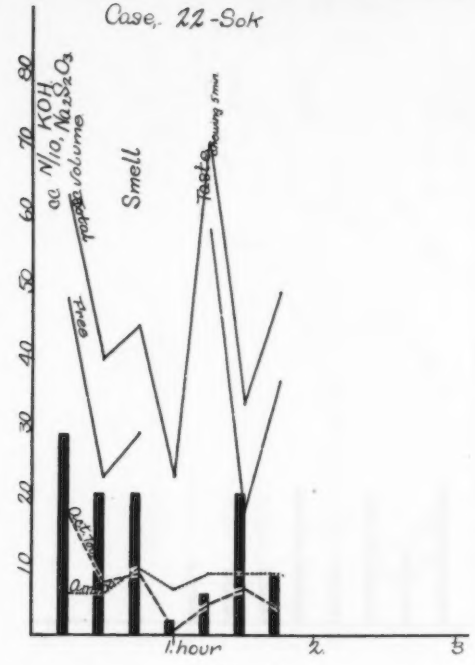
Case. 22-Sok
cc. N/10. KOH
Na2S2O3
Smell
Taste
1 hour
2
3

FIG. 15.


Case. 23-Swe
cc. N/10. KOH
Na2S2O3
Smell
Taste
1 hour
2
3

FIG. 16.

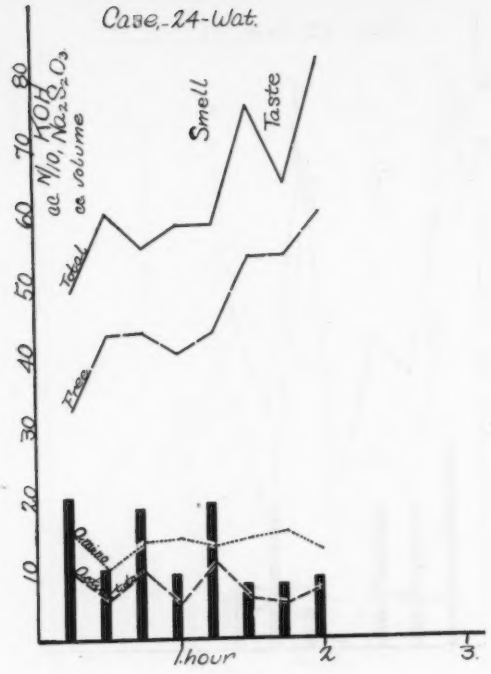
Case,-24-Wat.
cc N/10 KOH, $Na_2S_2O_3$ as volume
Smell
Taste
Total
Free
Amino
Org.
1.hour
2
3.

FIG. 17.

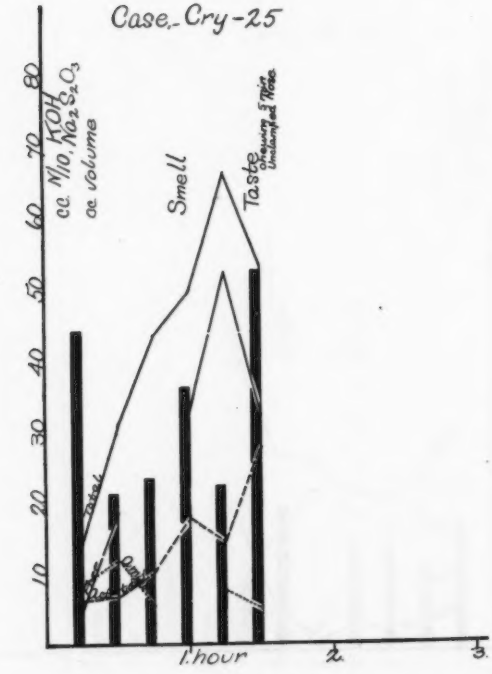
Case,-Cry-25
cc N/10 KOH, $Na_2S_2O_3$ as volume
Smell
Taste
Total
1.hour
2
3.

FIG. 18.

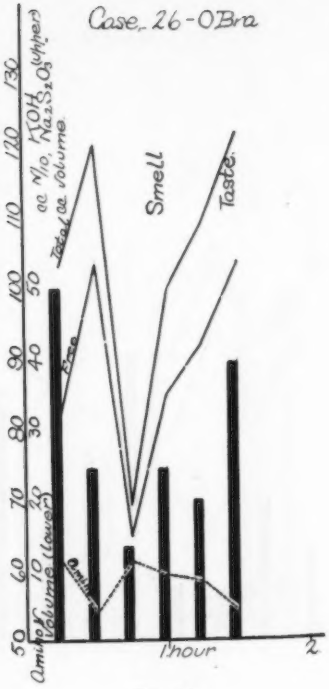
Case 26 - OBra
Smell
Taste
Free
1 hour
2

FIG. 19.

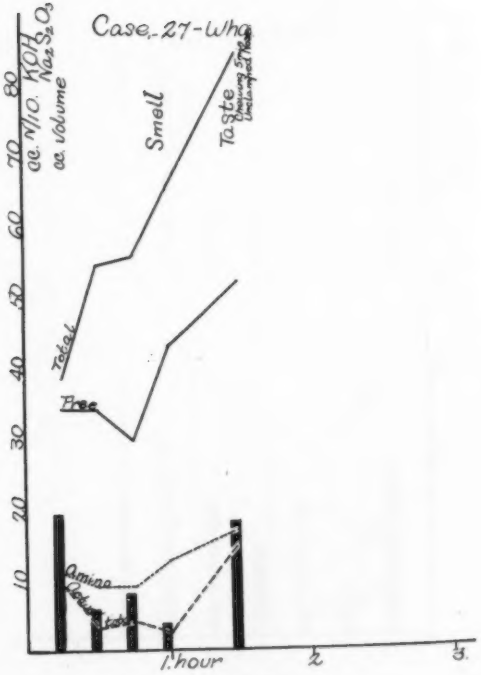
Case 27 - Wha
Smell
Taste
Free
1 hour
2
3

FIG. 20.

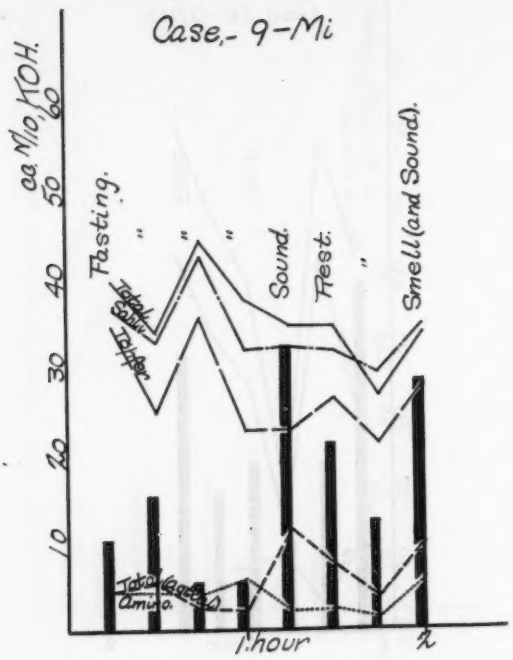
Case,- 9-Mi
cc. N/10, KOH.
10
20
30
40
50
60
Fasting.
"
"
"
Sound.
Rest.
"
Smell (and Sound).
1 hour
2

FIG. 21.

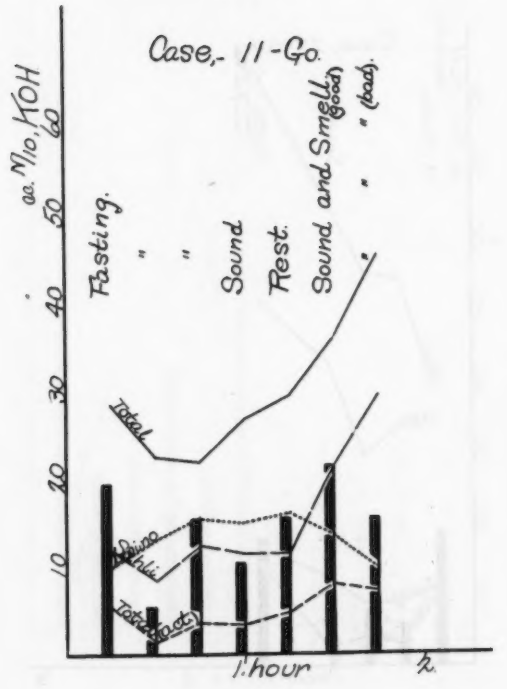
Case,- 11-Go.
cc. N/10, KOH
10
20
30
40
50
60
Fasting.
"
Sound
Rest.
Sound and Smell
"
"
(loud).
Total
1 hour
2

FIG. 22.

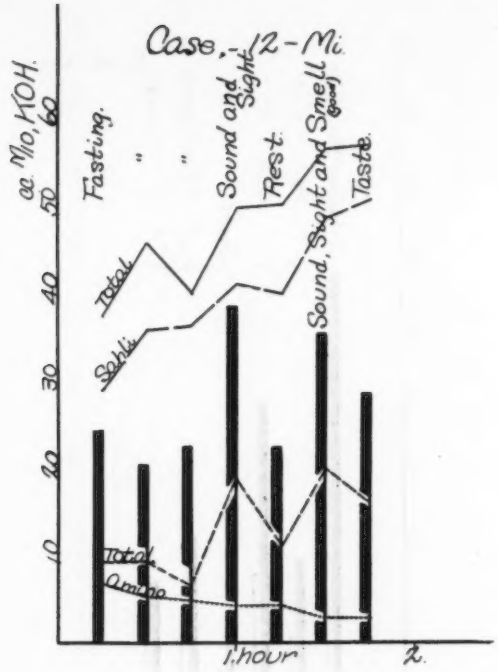
Case. - 12 - Mi.
cc N/10 KOH.
Fasting.
Sound and Sight
Rest.
Sound, Sight and Smell
Taste.
Total
Schli
Total
Amino
1. hour
2.

FIG. 23.

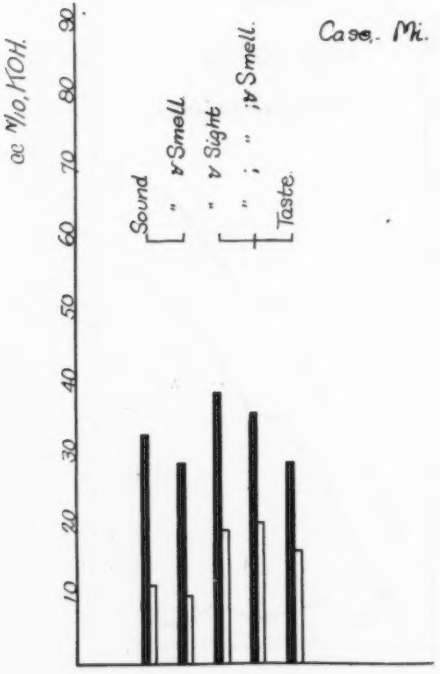
Case. - Mi.
cc N/10 KOH.
Sound
" & Smell
" & Sight
" " Smell.
" "
Taste.

FIG. 24.

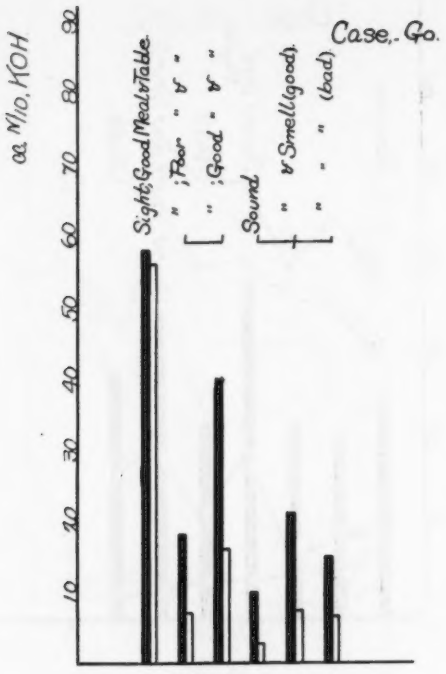
Case,-Go.
cc N/10, KOH
Sight; Good Meal v Table.
" ; Poor " v "
" ; Good - v "
Sound
" v Smell (good).
" - " (bad)
10
20
30
40
50
60
70
80
90

FIG. 25.

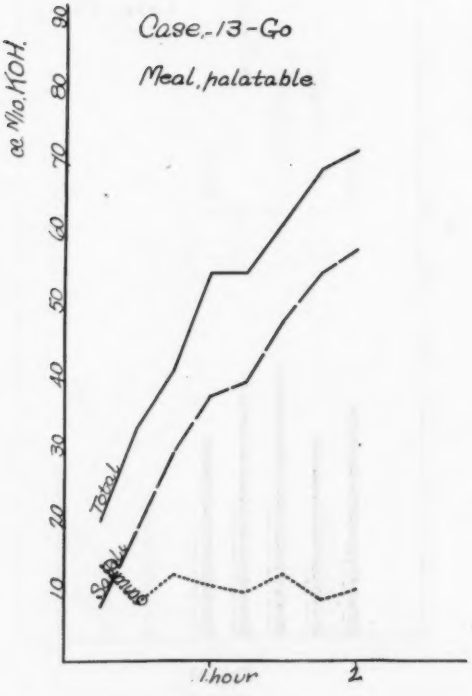
Case,-13-Go
Meal, palatable
cc N/10 KOH.
Total
Sounds
10
20
30
40
50
60
70
80
90
1 hour
2

FIG. 26.

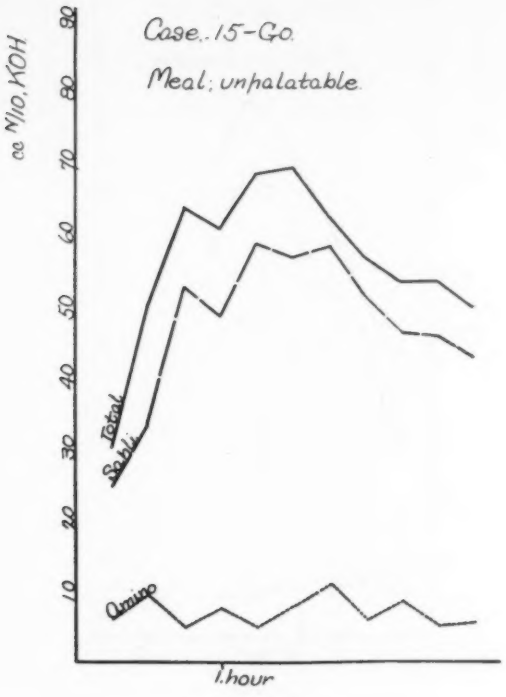
Case. 15-Go.
Meal; unpalatable.
cc N/10 KOH
80
70
60
50
40
30
20
10
Total
Sabli
Amino
1 hour

FIG. 27.

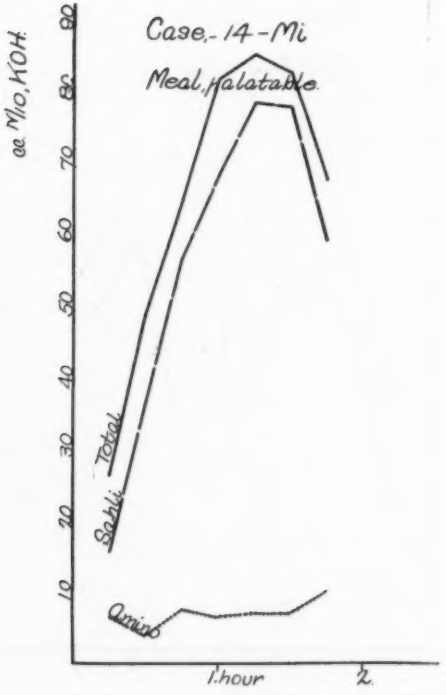
Case. 14-Mi
Meal, palatable.
cc N/10 KOH
80
70
60
50
40
30
20
10
Total
Sabli
Amino
1 hour
2

FIG. 28.

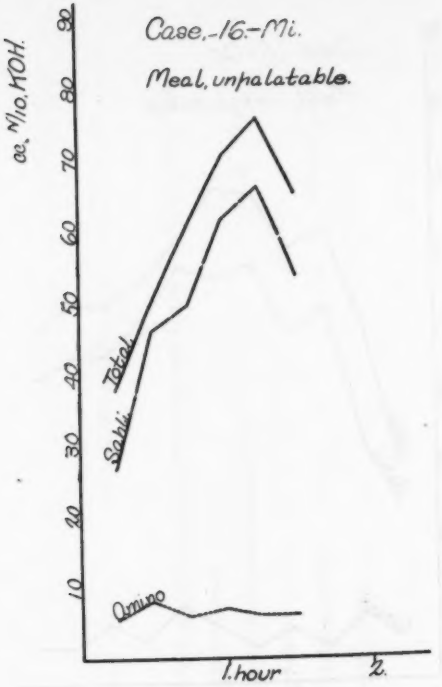
Case,-16.-Mi.
Meal, unpalatable.
cc. N/10. KOH.
10
20
30
40
50
60
70
80
90
Total
Sabli
Amino
1. hour
2.

FIG. 29.

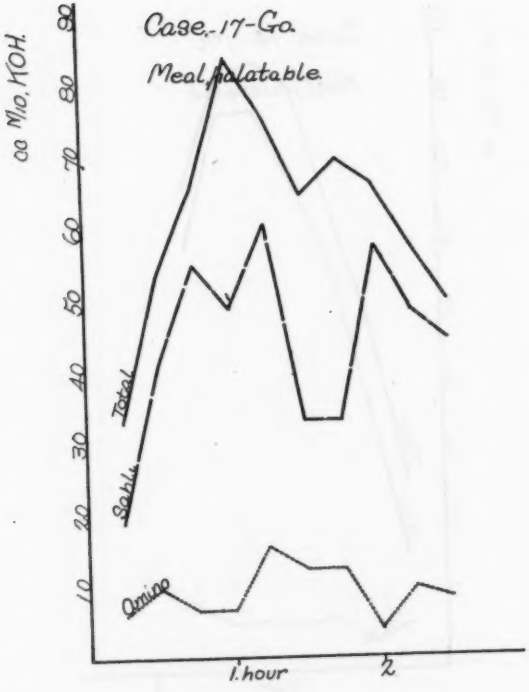
Case,-17-Ga.
Meal, palatable.
cc M/10. KOH.
10
20
30
40
50
60
70
80
90
Total
Sabli
Amino
1. hour
2

FIG. 30.

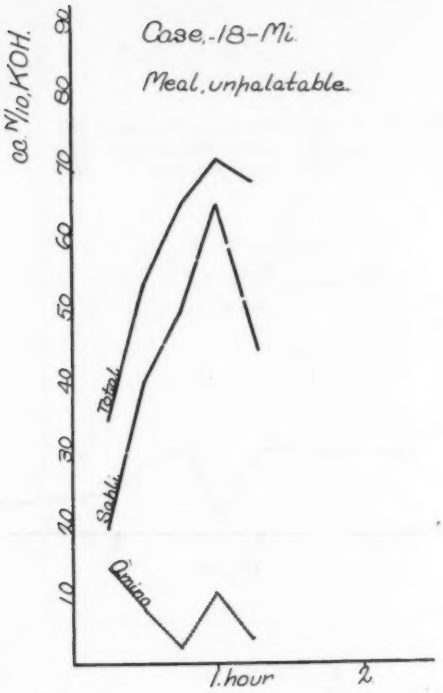
Case.-18-Mi.
Meal, unpalatable.
cc. N/10, KOH.
10
20
30
40
50
60
70
80
90
Total
Soluble
Amino
1 hour
2

FIG. 31.

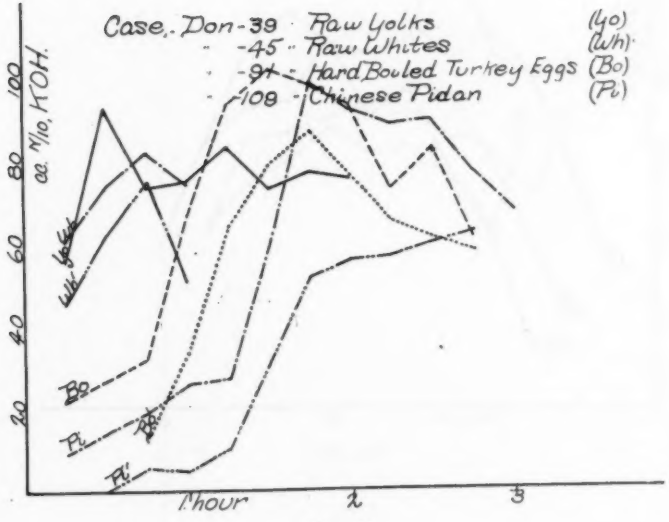
Case.-Don-39 - Raw Yolks (Yo)
-45 - Raw Whites (Wh)
-91 - Hard Boiled Turkey Eggs (Bo)
-108 - Chinese Pidan (Pi)
cc. N/10, KOH.
20
40
60
80
100
Yo
Wh
Bo
Pi
1 hour
2
3

FIG. 32.

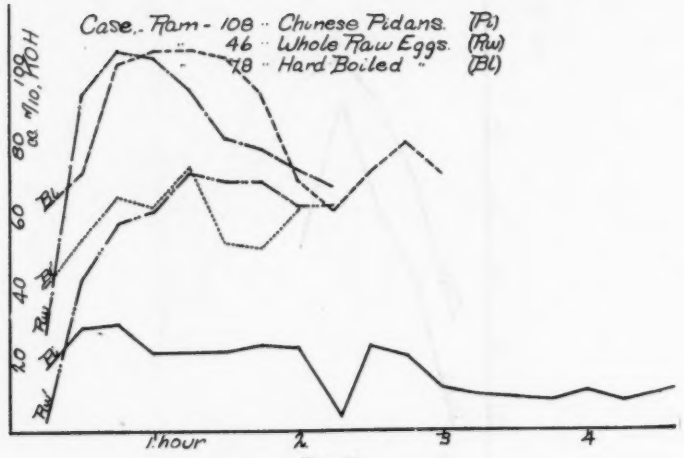
Case. Ham - 108 " Chinese Pidans (Pi)
" 46 " Whole Raw Eggs (Rw)
" 18 " Hard Boiled " (Bl)
cc N/10 NaOH
100
80
60
40
20
Bl
Bl'
Rw
Rw'
Pi
Pi'
1 hour
2
3
4

FIG. 33.

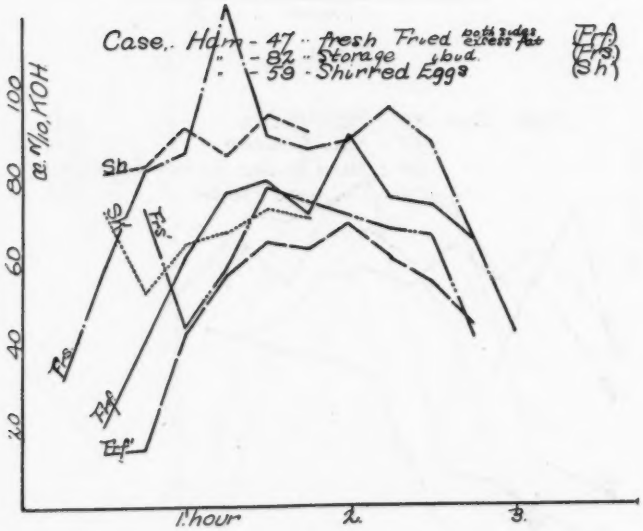
Case. Ham - 47 " fresh Fried both sides cut off (Fr)
" - 82 " Storage " (Fr3)
" - 59 " Shirred Eggs (Sh)
cc N/10 NaOH
100
80
60
40
20
Sh
Sh'
Fr3
Fr3'
Fr
Fr'
1 hour
2
3

FIG. 34.

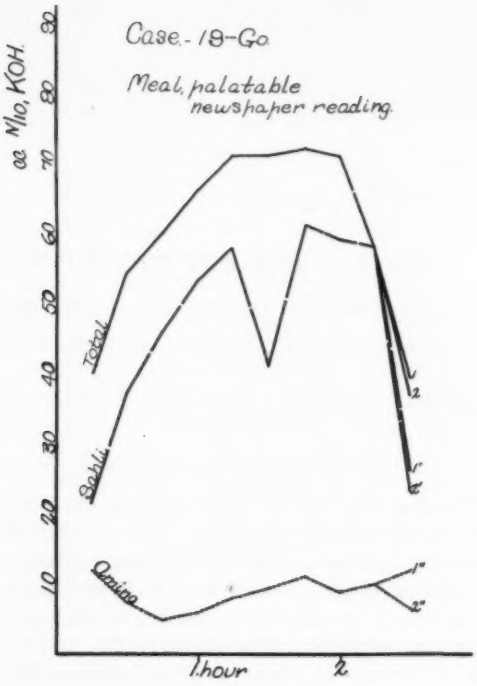
Case.-18-Go
Meal, palatable
newspaper reading.
cc N/10, KOH.
10
20
30
40
50
60
70
80
90
Total
Soluble
Quinine
1 hour
2

FIG. 35.

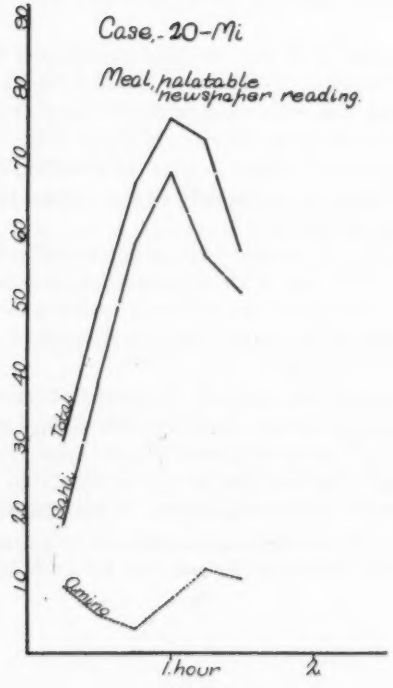
Case.-20-Mi
Meal, palatable
newspaper reading.
10
20
30
40
50
60
70
80
90
Total
Soluble
Quinine
1 hour
2

FIG. 36.

GASTRIC RESPONSE TO FOODS[1]

XI. The Influence of Tea, Coffee and Cocoa upon Digestion

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Many experiments have been made to determine the effects of tea and coffee on peptic digestion in vitro. The object of the series of experiments reported here was somewhat different, namely, to determine in what way the responses of the stomachs of normal human subjects to mixed meals would be affected by adding to such meals equal volumes of water, tea, coffee or cocoa—hot or cold—and with or without the addition of cream or sugar. Certain observations relative to changes in pulse rate and other symptoms following ingestion of these beverages were also made, but the experiments were not complete in this respect.

We are not familiar with any previous experiments of the type carried out by us. Penzoldt (1), however, studied the evacuation of 200 cc. portions of water, tea, coffee and cocoa on a single subject and found them to leave the stomach as follows: Water, 1 hour, 15 minutes; tea, 1 hour, 30 minutes; black coffee, 1 hour, 45 minutes; coffee with 5 cc. cream, 2 hours, 15 minutes; cocoa with water, 1 hour, 45 minutes; cocoa with milk, 2 hours, 30 minutes.

Our experiments were carried out upon normal medical students. They reported at 8:00 a.m. without breakfast, and without removal of residuum they were given the following uniform meal, either alone or with the beverage to be tested which was drunk during the course of ingestion of the other foods.

Test meal: Scraped beef, grilled, 75 grams; mashed potatoes, 50 grams; toast, 40 grams; butter, 15 grams; salt, 1½ to 2 grams as desired.

The tea, coffee and cocoa used were of good quality and prepared of a moderate strength by the dietitian of the hospital, exactly as used in the regular dietary of the institution. Where sugar was used, four

[1] The expenses of this investigation were defrayed by grants from Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

lumps of sugar per liter were given. In the case of cream approximately 15 cc. of a 20 per cent cream were used. No milk or added sugar were used in preparing the cocoa, which was made up according to the directions on the container.

As soon as the meal was finished the subjects were made to swallow the Rehfuss stomach tubes. From 5 to 8 cc. of the stomach contents were aspirated at 15-minute intervals for 1½ hours. The subjects were then allowed to attend a class and returned 1½ hours later (after a total experimental period of 3 hours). Then two samples were withdrawn at 10-minute intervals, and finally the stomach was completely emptied, this being checked by a lavage of 150 cc. of water.

Total and free acidities were determined by titration and pepsin by the Mett method. The volumes of contents at the end of the 3-hour period were measured. The pulse rate was also determined at intervals and subjects reported any unusual symptoms.

Tests were carried out in which the following beverages were added to the uniform test meals:

(1) Cold water, 1 liter, 15°C.
(2) Hot coffee, plain, 1 liter, 45–50°C.
(3) Hot coffee, with sugar, 1 liter, 45–50°C.
(4) Hot coffee, with cream, 1 liter, 45–50°C.
(5) Hot coffee, cream and sugar, ½ liter, 45–50°C.
(6) Cold tea, plain, 1 liter, 13°C.
(7) Hot tea, plain, 1 liter, 50°C.
(8) Hot cocoa, plain, 1 liter, 57°C.
(9) Hot cocoa, plain, ½ liter, 57°C.

Thirty-seven experiments were carried out on four different subjects, duplicates being made on each subject with the basal diet alone. The results are charted in figures 1 to 34. The results with regard to evacuation time are summarized in figure 34.

INFLUENCE OF BEVERAGES ON THE ACID RESPONSE OF THE STOMACH

Influence of cold water on the acid response. The diet alone containing, as it does, considerable meat gives rise to the rapid development of a relatively high acidity, much of which is represented by combined acid (figs. 1, 10, 19 and 26). As no liquid whatever was taken with the meals in these cases and in the early part of digestion the absorption of gastric juice by the meat was pronounced, a uniform mixture

was not at once obtained in the stomach; and the samples withdrawn varied somewhat with the exact location of the tip in the stomach. For this reason the duplicate curves do not coincide, in spite of the fact that digestion follows the same course and is concluded after the same lapse of time. Where a moderate amount of liquid is present or the combining power of the food less, it is possible to sample uniformly and to obtain very similar curves with the same food eaten at different times. For this reason the experiments on different beverages are, with regard to acid response, more comparable with each other than with the diet lacking liquid.

The curves of acidity, especially total and combined acidity, appeared to rise somewhat more quickly and to a generally higher level with the diet alone than with the added liter of cold water. As the evacuation time was practically unaffected and the acidities still high following the high water ingestion, the water cannot be said to have hindered digestion and must have given rise to a considerable stimulation of acid secretion or been rapidly emptied in large measure, or more probably both.

In consideration of the results of these tests it must be constantly borne in mind that unusual amounts of the various beverages were given. This was intentionally done in order that any untoward influences might be accentuated.

Influence of coffee on the acid response. Hot coffee, plain, that is without cream or sugar and at a temperature of 45 to 50°C. was given to four subjects. The acid responses are charted in figures 3, 12, 21 and 27. In the first three cases a liter of the coffee was taken; in case 27, however, only half a liter. The charts show in a general way that the acid responses were very similar to the results obtained with cold water, and not very different from those obtained with the basic meal by itself, although not attaining quite the heights of total acidity given by the latter. There was no distinct indication that the coffee inhibited the secretion of the gastric juice.

Sugar was added to the coffee in two cases (see figs. 4 and 22). Comparisons of the acid responses with those of the same individuals taking plain coffee (see figs. 3 and 21) show that the sugar depressed considerably the acid response. It has been clearly demonstrated in this laboratory that sugar depresses gastric secretion; and it is, therefore, improbable that the low acid values in the earlier period of digestion in the cases where sugar was added to the coffee, are due merely to the slower evacuation of the beverage.

The addition of moderate amounts of cream (15 cc. of 20 per cent cream to the liter) was tried out in two cases (compare figs. 12 and 13 and figs. 27 and 28). It will be noted that the acid response was not demonstrably influenced by such addition.

Hot coffee with both cream and sugar was given in four cases (see figs. 5, 14 and 29). The results indicate that with the addition of cream and sugar the acid response was higher than where sugar alone was used, and more nearly approached that of the plain coffee. This may perhaps be explained by the fact that the coffee was more appetizing with additions of cream and sugar, provoking a more marked appetite secretion and also leading to more rapid evacuation.

Influence of tea on the acid response. One liter of cold tea (12° to 13°C.) was given during the course of the standard meal to each of these subjects (see figs. 6, 15 and 30). If the acid responses are compared with those of the same subjects to cold water or plain hot coffee, it will be noted that they are very similar, although one subject showed a slightly higher curve with tea than with water or coffee.

Hot tea (50°C.) was also given to three subjects (see figs. 7, 16, 24 and 31). With slight variations, generally in favor of the hot tea, the results obtained were not distinctly different from those with water, coffee or cold tea.

Influence of cocoa on the acid response. That cocoa when taken in amounts of 1 liter at a meal has a depressing action on the development of gastric acidity is clearly apparent from the results on our four subjects as charted in figures 8, 17, 25 and 32. In each case the intragastric acidity rises slowly as compared with either cold water, hot coffee or hot or cold tea. This may be due to several causes. Undoubtedly the sugar content depresses secretion and delays evacuation as sugar solutions have been clearly shown to do. The higher fat content of cocoa may play a part as well as the presence of other constituents.

In three cases the amount of cocoa drunk was reduced to one-half liter. The depressing action of cocoa on the development of acidity may be noted in these cases (see figs. 9, 18 and 33). In general this depression is about equal to that of one liter of tea or coffee.

INFLUENCE OF WATER, TEA, COFFEE AND COCOA ON THE EVACUATION TIME OF THE NORMAL HUMAN STOMACH

The influence of the beverages studied on the evacuation time of the stomach is summarized in figure 34, which gives the volumes of the gastric contents at the time of complete removal (approximately 3 hours after ingestion). The evacuation of the stomach was not appreciably delayed by the drinking during the meal of 1 liter of cold water; hot coffee, plain; hot coffee with cream; hot coffee with cream and sugar; cold tea, or hot tea; nor were there any distinct differences between the evacuation periods of any of these beverages. The addition of sugar alone to coffee did, however, delay evacuation distinctly in both cases. Most striking, however, was the pronounced delay in evacuation caused by cocoa, this being noted in every case, even where half amounts only were given. For example, in one case the gastric contents at three and one-half hours measured, following the meal alone, 124 cc.; following hot coffee with cream and sugar, 110 cc.; and after cocoa, 465 cc.

Temperature appeared to have little influence on evacuation time, meals including cold tea leaving the stomach in the same time as those including equal volumes of hot tea; nor did cold water delay evacuation.

INFLUENCE OF TEA, COFFEE AND COCOA ON THE PEPTIC RESPONSE

Peptic activities were determined by the Mett method. In figure 35 are illustrated the values obtained after the uniform meals had been given with water, tea, coffee and cocoa respectively. The only striking differences in the development of peptic activity noted were the low values found after the ingestion of coffee with sugar and especially after the ingestion of cocoa. These low values may be due to the depression of the secretory mechanism by the sugar and other substances present in cocoa as well as to retention of the ingested beverages.

INFLUENCE OF TEA, COFFEE AND COCOA ON THE PULSE RATE

The pulse rate was also followed in each case, although not as systematically as would be necessary for exact comparisons of the effects of the different beverages upon the heart beat. Tea, and particularly coffee, did, however, bring about in liter quantities a pronounced acceleration of the heart beat in the first half-hour, one subject showing an

increase to 150 and 160 beats per minute after hot tea and coffee respectively. There was some evidence that coffee brought about a more rapid and pronounced acceleration of the heart beat than the other beverages. From its slow evacuation, cocoa might be expected to produce a less rapid stimulation. An attempt (2) has been made to account for the more rapid action of coffee as compared with tea by showing that in coffee the alkaloid exists as caffeotannic acid soluble in cold water and not readily precipitated, while tea contains a caffeine tannate soluble in hot but not cold water and which is readily precipitated by acid (hence presumably by the gastric juice), going readily back in solution in an alkaline medium (such as that of the pancreatic juice). While coffee is usually about a 6 per cent decoction and tea about $1\frac{1}{4}$ per cent, it must be borne in mind that tea contains 3 to 4 per cent of caffeine and coffee about 1 per cent.

Other systemic effects of tea, coffee and cocoa. Tea and coffee in the amounts taken gave rise in our subjects to nervousness, vasomotor relaxation, sweating, tremors, headaches, dizziness and sleeplessness, in some cases to a marked degree. Three of our subjects were unaccustomed to the use of coffee, and the fourth never drank more than one cup at a meal. It is, therefore, probable that the symptoms noted were of a somewhat more aggravated character than would be found in the case of persons habitually drinking much tea or coffee. There is no question, however, that tea and coffee may have a marked effect upon the circulation, and that they are in no wise to be considered as beverages to be used in an unrestricted manner.

Cocoa did not give rise to nervous or vasomotor symptoms to anything like the same extent as tea and coffee. This may in part have been due to slow absorption. In all cases there was a feeling of fulness after cocoa and a lack of hunger, which may be readily explained by the prolonged retention of this beverage in the stomach.

DIURESIS AFTER TEA AND COFFEE DRINKING

The urine secretion of our subjects was measured during the period immediately following the ingestion of the test meals with tea, coffee and cocoa. The volumes of urine eliminated are indicated by charts 36, 37 and 38. As much as 866 cc. of urine was excreted within an hour and a half after 1 liter of tea was given, and the values after coffee drinking were of the same order. It is clear, therefore, that these beverages left the stomach quickly, were rapidly absorbed, and the excess

water soon eliminated. Coffee with sugar resulted in less rapid elimination, and the secretion after cocoa was low in volume, as might be expected from the delayed gastric evacuation. The specific gravities of the urines during the period of most rapid elimination varied from 1.004 to 1.001 or less, indicating a very dilute secretion.

SUMMARY AND CONCLUSIONS

A study was made of the influence of water, tea, coffee and cocoa upon the gastric digestion of a uniform mixed meal as measured by the acid responses and evacuation times.

Evacuation of the stomach was not appreciably delayed by the drinking of 1 liter of cold water, cold or hot tea, hot coffee, either plain, with cream or with cream and sugar. The addition of sugar alone to coffee delayed evacuation.

Cocoa in 1 liter quantities markedly delayed evacuation. To a less extent this was true of half-liter volumes.

One liter quantities of water, hot or cold tea, hot coffee, plain or with cream, delayed somewhat the rise of the level of intragastric acidity as compared with the basal meal alone. As high acidities and normal evacuation were, however, attained these beverages must have stimulated gastric secretion, been rapidly evacuated, or more probably both.

Coffee with sugar alone delayed the development of gastric acidity. Coffee with sugar and cream had less effect.

Cocoa delayed distinctly the development of intragastric acidity.

One liter quantities of tea and coffee gave rise to marked acceleration of the heart beat, to vasomotor relaxation, tremors and other nervous symptoms.

Cocoa did not produce these effects but brought about a feeling of fulness at the stomach.

Urine secretion during the first 90 minutes after tea or coffee ingestion varied from 550 to 866 cc., after cocoa from 125 to 372 cc.

The authors desire to thank the students who kindly served as the subjects of these tests.

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(1) Penzoldt: Deutsch. Arch. klin. Med., 1893, xiv, 535.
(2) Lancet, 1911, i, 46; ii, 1573.

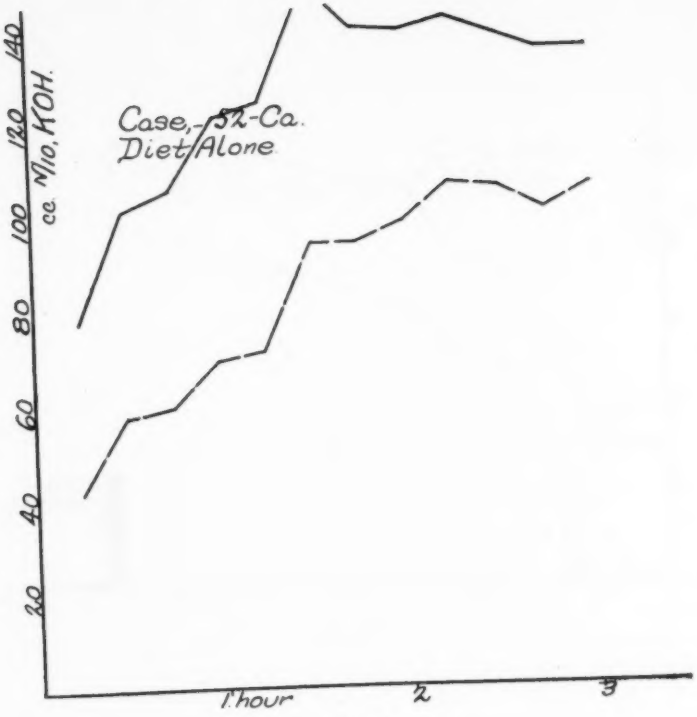
Case, 52-Ca.
Diet Alone
cc. N/10. KOH.
140
120
100
80
60
40
20
1 hour
2
3

FIG. 1

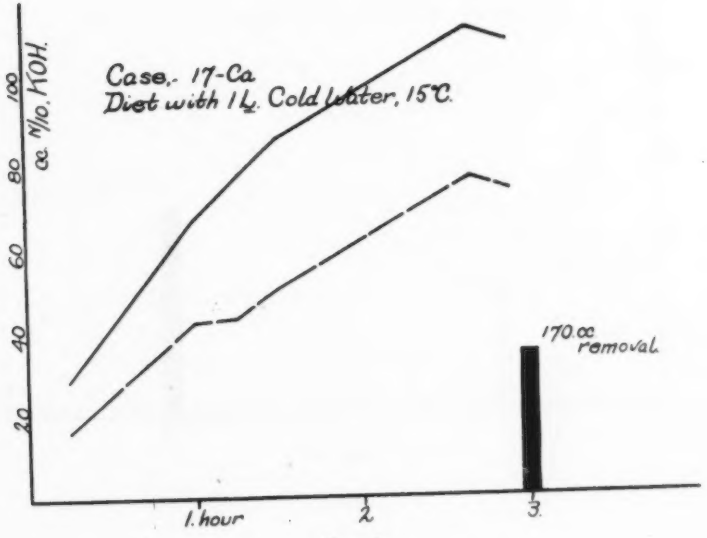
Case, 17-Ca
Diet with 1L. Cold water, 15°C.
cc. N/10. KOH.
100
80
60
40
20
170 cc removal.
1 hour
2
3

FIG. 2

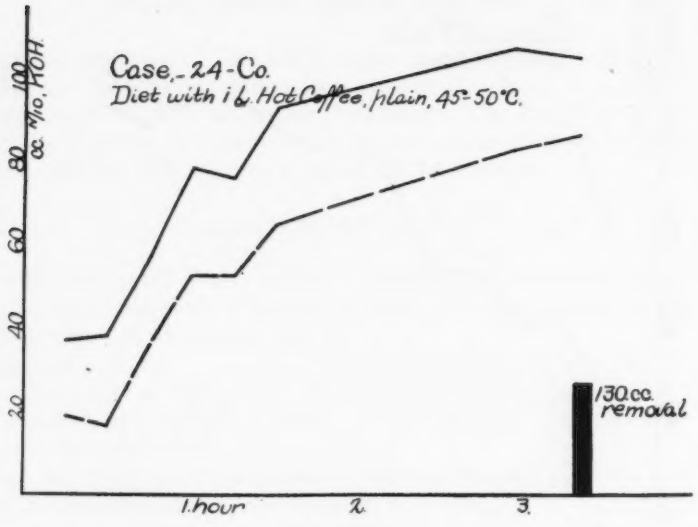
Case.- 24-Co.
Diet with 1 l. Hot Coffee, plain, 45°-50°C.
cc N/10 KOH
100
80
60
40
20
130 cc. removal
1. hour
2.
3.

FIG. 3

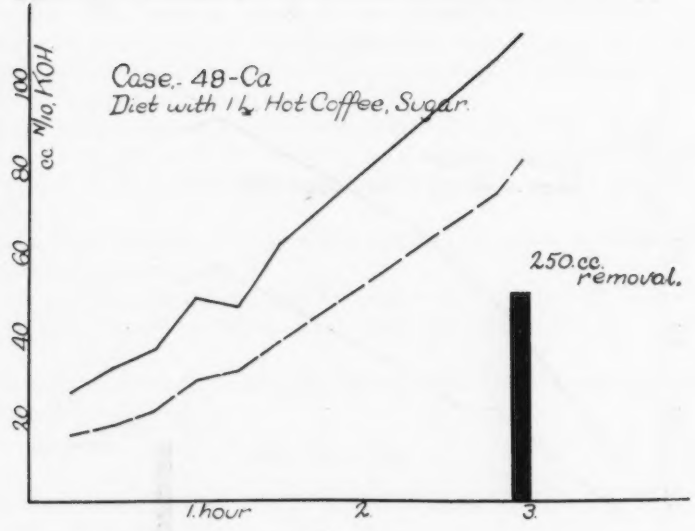
Case.- 48-Ca
Diet with 1 l. Hot Coffee, Sugar.
cc N/10 KOH
100
80
60
40
20
250 cc. removal.
1. hour
2.
3.

FIG. 4

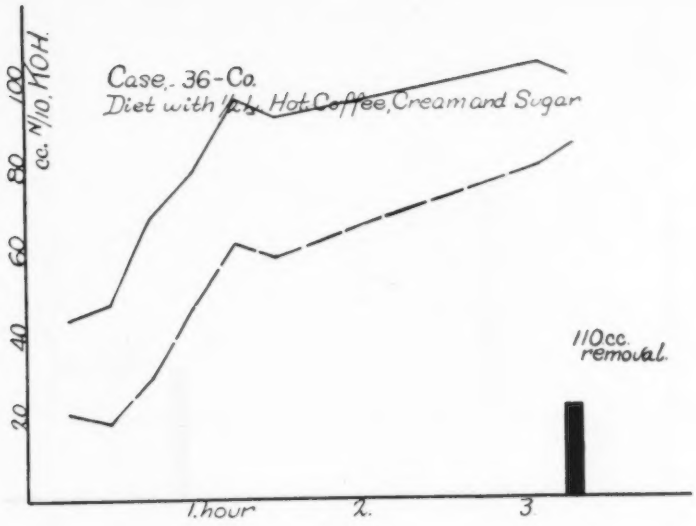
Case,- 36-Ca.
Diet with ½ lb. Hot Coffee, Cream and Sugar
cc. N/10. KOH.
100
80
60
40
20
110cc.
removal.
1. hour
2.
3

FIG. 5

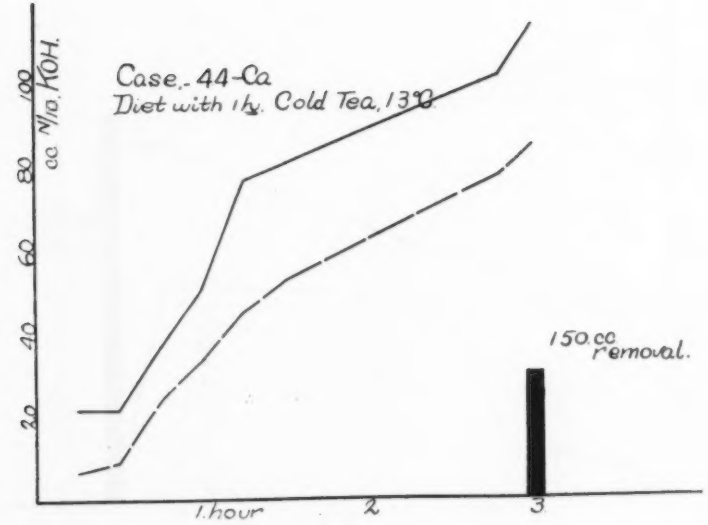
Case,- 44-Ca
Diet with 1½. Cold Tea, 13°
cc N/10. KOH.
100
80
60
40
30
150.cc
removal.
1. hour
2
3

FIG. 6

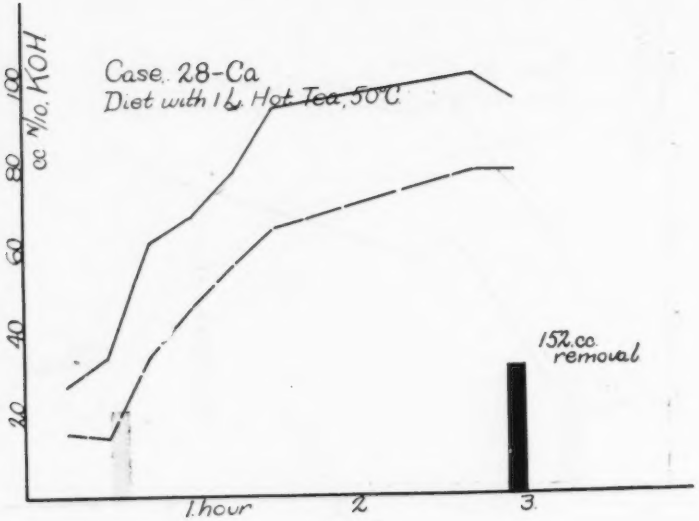
Case. 28-Ca
Diet with 1 L. Hot Tea, 50°C.
cc N/10. KOH
100
80
60
40
20
152 cc. removal
1 hour
2
3

FIG. 7

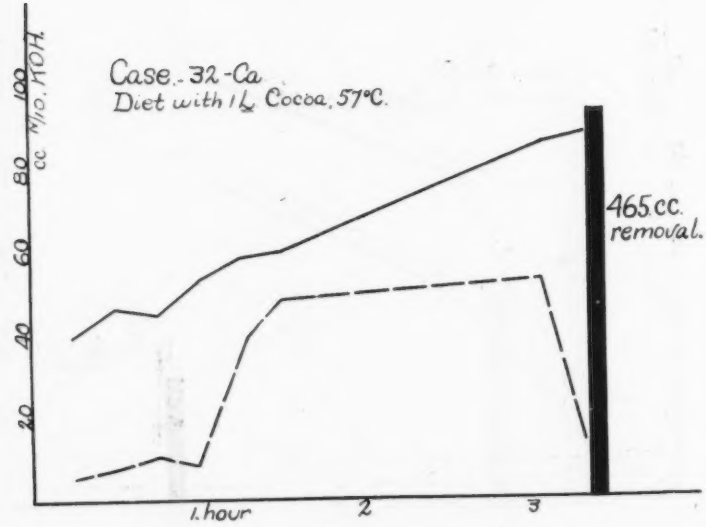
Case. 32-Ca
Diet with 1 L. Cocoa, 57°C.
cc N/10. KOH
100
80
60
40
20
465 cc. removal.
1 hour
2
3

FIG. 8

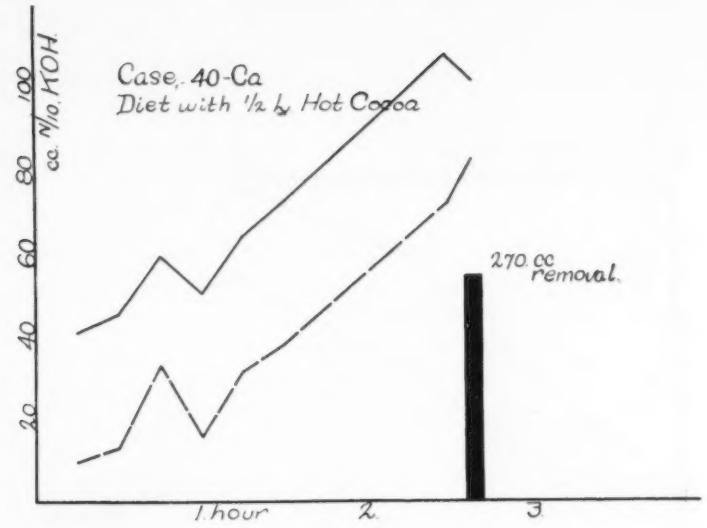
Case, 40-Ca
Diet with 1/2 L. Hot Cocoa
cc. N/10. KOH.
100
80
60
40
20
270 cc removal.
1 hour
2
3

FIG. 9

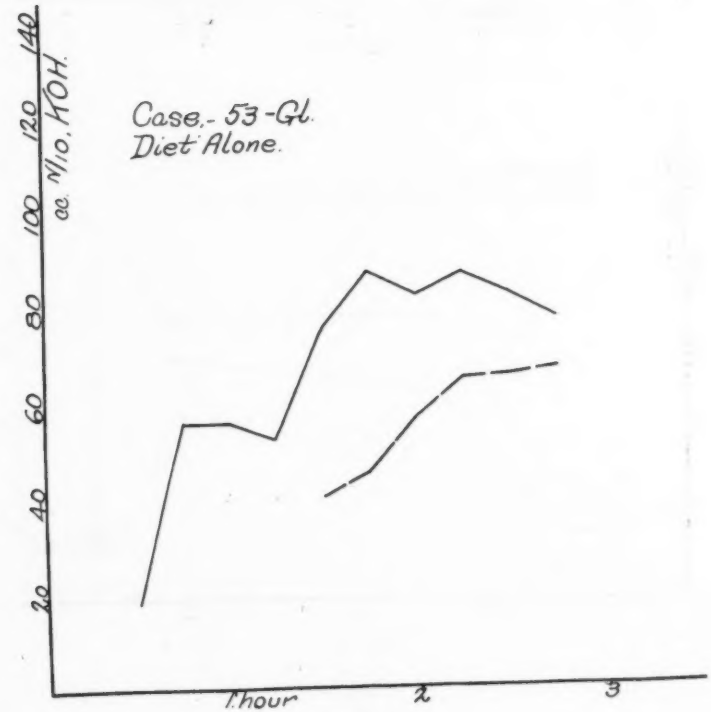
Case,- 53-Gl.
Diet Alone.
cc. N/10. KOH.
140
120
100
80
60
40
20
1 hour
2
3

FIG. 10

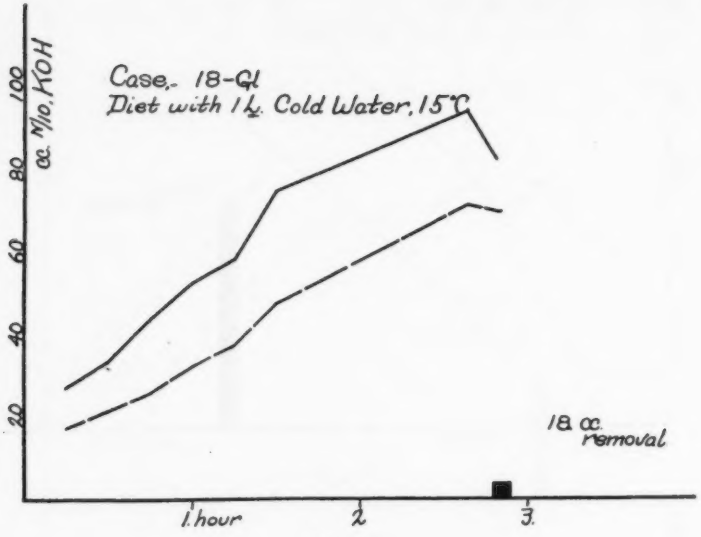
Case. 18-Gl
Diet with 1/4. Cold Water, 15°C
cc. N/10. KOH
100
80
60
40
20
18 cc. removal
1 hour
2
3

FIG. 11

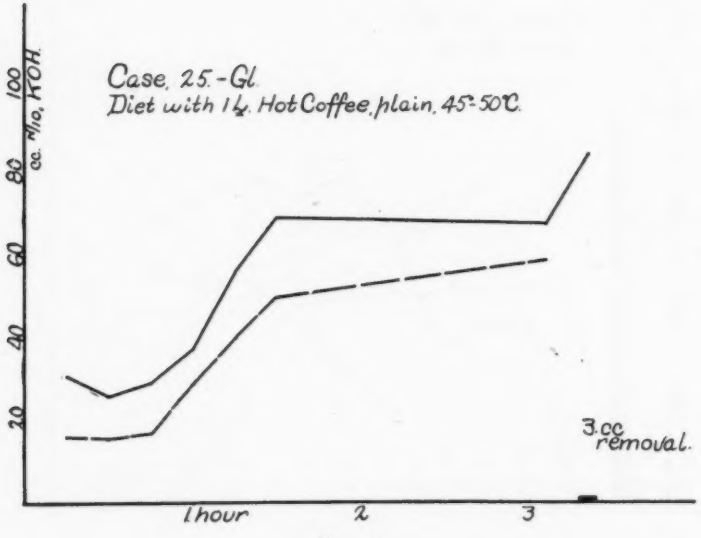
Case. 25.-Gl
Diet with 1/4. Hot Coffee, plain, 45°-50°C.
cc. N/10. KOH.
100
80
60
40
20
3 cc. removal.
1 hour
2
3

FIG. 12

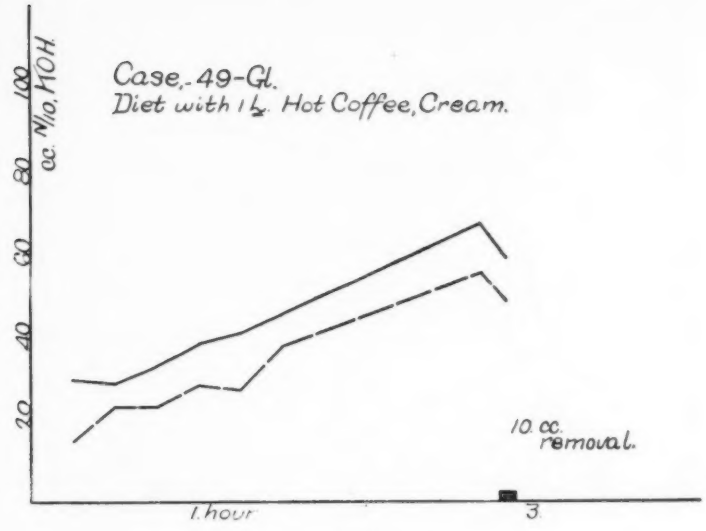
Case, 49-Gl.
Diet with 1 lb. Hot Coffee, Cream.
cc. N/10. KOH.
100
80
60
40
20
10 cc. removal.
1 hour
3

FIG. 13

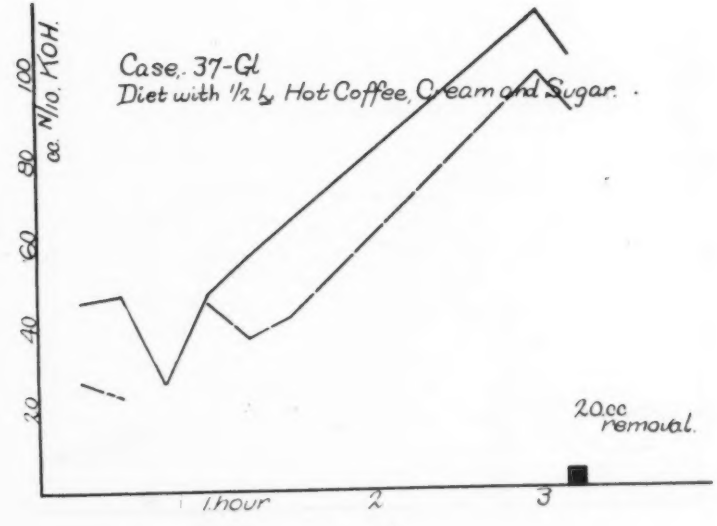
Case, 37-Gl
Diet with 1/2 lb. Hot Coffee, Cream and Sugar.
cc. N/10. KOH.
100
80
60
40
20
20 cc. removal.
1 hour
2
3

FIG. 14

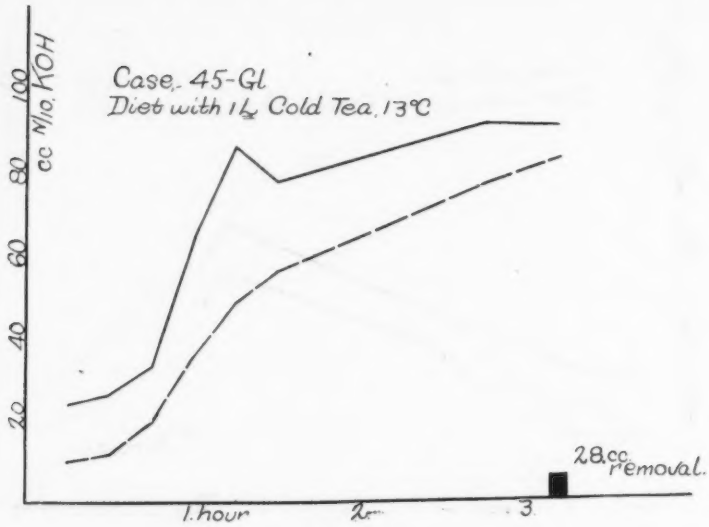
Case. 45-Gl
Diet with 1 L. Cold Tea, 13°C
cc N/10 KOH
100
80
60
40
20
28 cc removal.
1 hour
2
3

FIG. 15

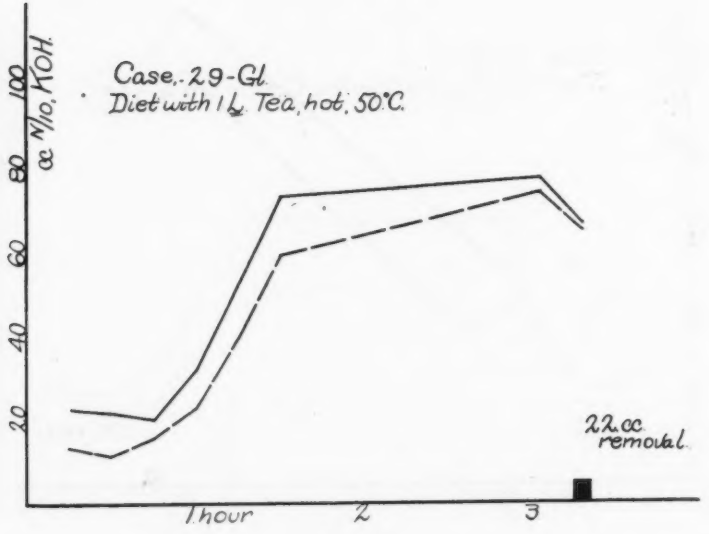
Case. 29-Gl
Diet with 1 L. Tea, hot, 50°C.
cc N/10 KOH
100
80
60
40
20
22 cc removal
1 hour
2
3

FIG. 16

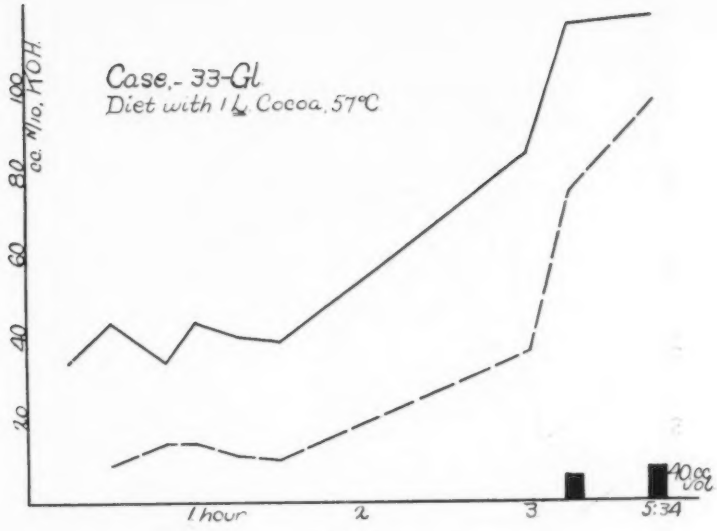
Case.- 33-Gl
Diet with 1 L. Cocoa. 57°C
cc. N/10. KOH
100
80
60
40
20
1 hour
2
3
5:34
40 cc
vol

FIG. 17

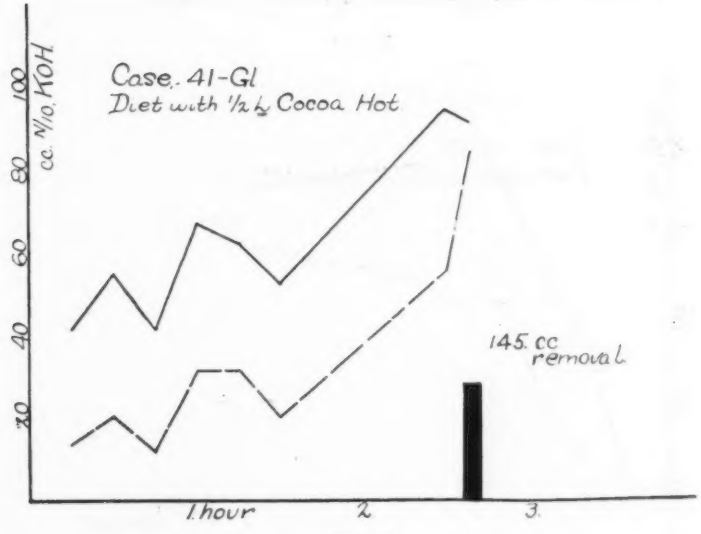
Case. 41-Gl
Diet with 1/2 L. Cocoa Hot
cc. N/10. KOH
100
80
60
40
20
145. cc
removal
1 hour
2
3

FIG. 18

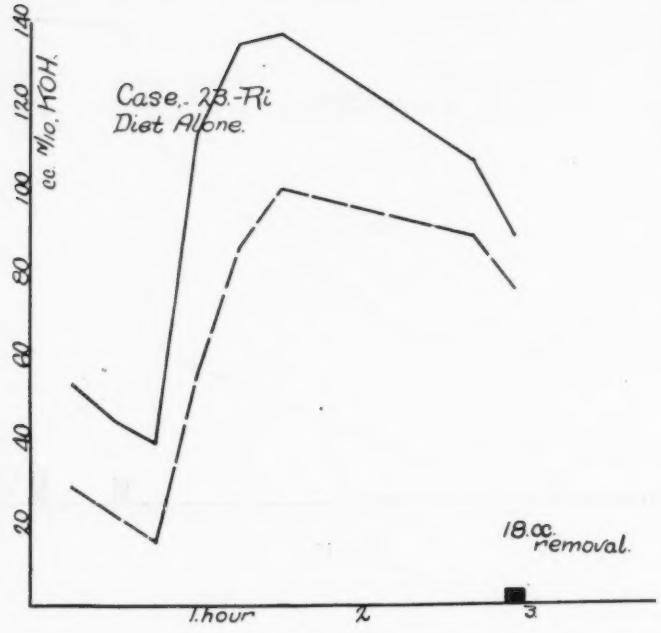
Case.- 23.-Ri
Diet Alone.
cc. N/10. KOH.
140
120
100
80
60
40
20
18.cc. removal.
1 hour
2
3

FIG. 19

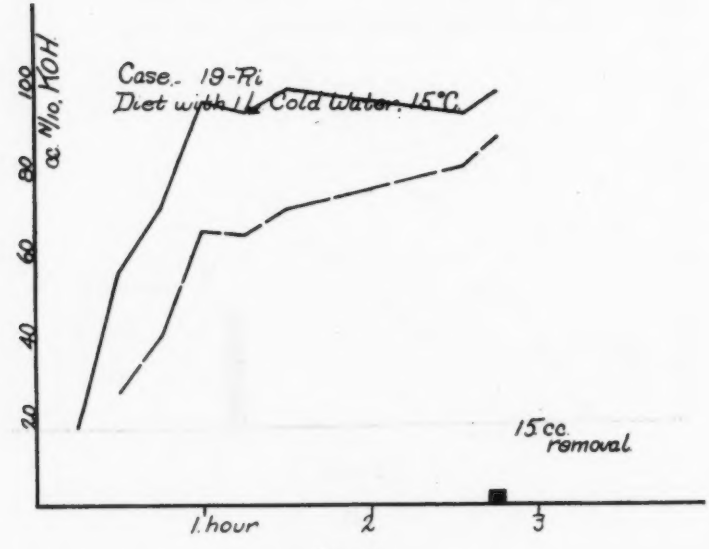
Case.- 19-Ri
Diet with 1/ Cold Water. 15°C.
cc. N/10. KOH
100
80
60
40
20
15 cc. removal.
1 hour
2
3

FIG. 20

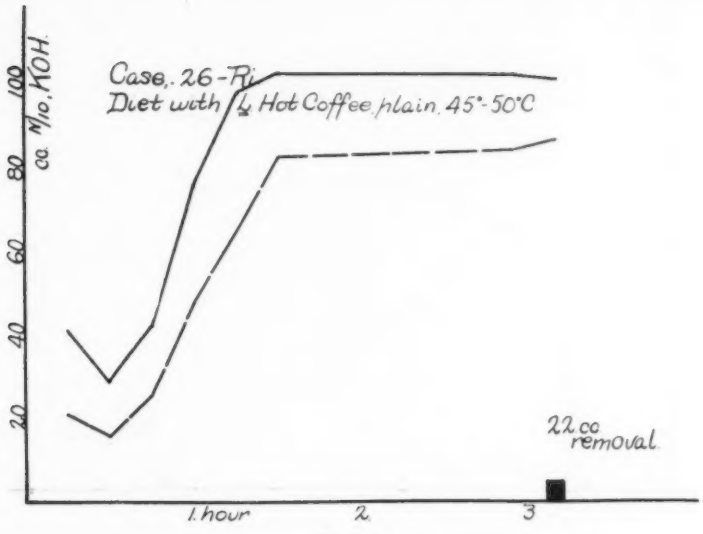
Case, 26-Ri
Diet with ½ Hot Coffee, plain, 45°-50°C
cc N/10 KOH
100
80
60
40
20
22 cc removal
1 hour
2
3

FIG. 21

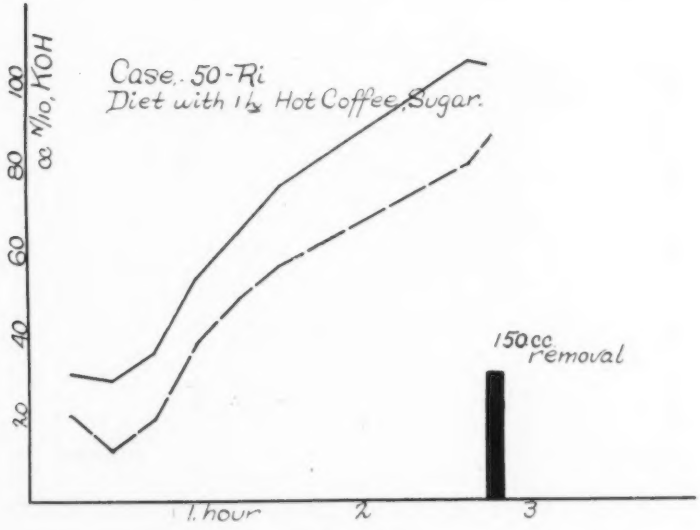
Case, 50-Ri
Diet with 1½ Hot Coffee, Sugar.
cc N/10 KOH
100
80
60
40
20
150 cc removal
1 hour
2
3

FIG. 22

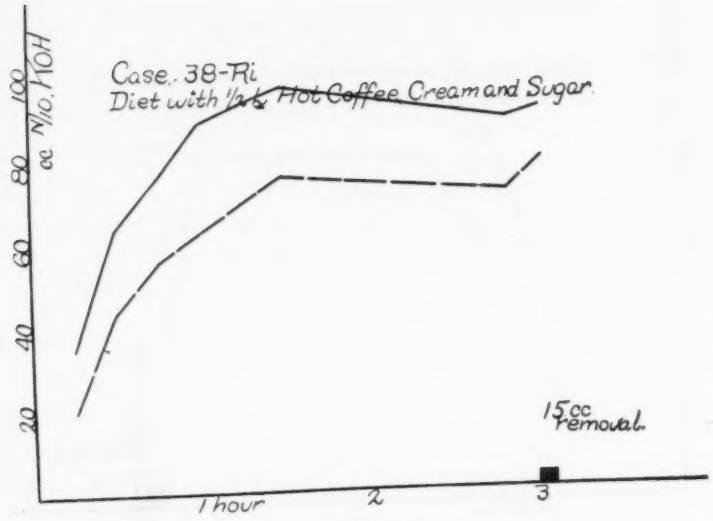
Case. 38-Ri
Diet with 1/2 lb. Hot Coffee Cream and Sugar.
cc N/10. KOH
100
80
60
40
20
15 cc removal.
1 hour
2
3

FIG. 23

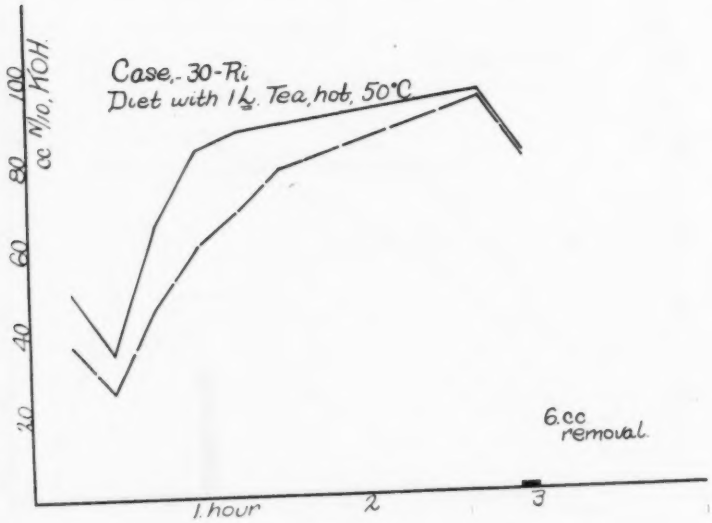
Case. 30-Ri
Diet with 1/2 Tea, hot, 50°C
cc N/10. KOH
100
80
60
40
30
6 cc removal.
1 hour
2
3

FIG. 24

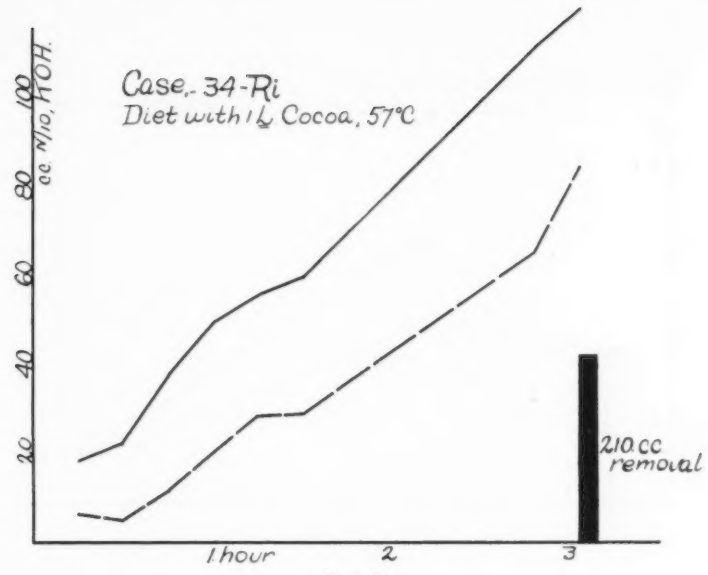
Case, 34-Ri
Diet with 1% Cocoa, 57°C
cc. N/10, KOH.
100
80
60
40
20
210 cc
removal
1 hour
2
3

FIG. 25

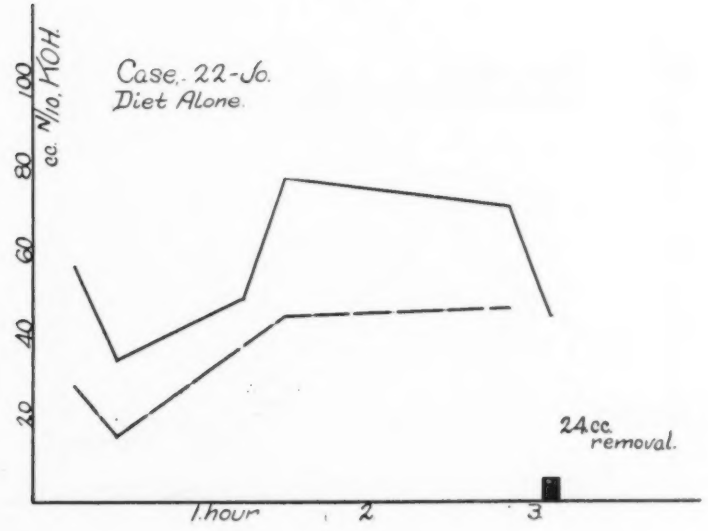
Case, 22-Jo.
Diet Alone.
cc. N/10, KOH
100
80
60
40
20
24 cc.
removal.
1 hour
2
3

FIG. 26

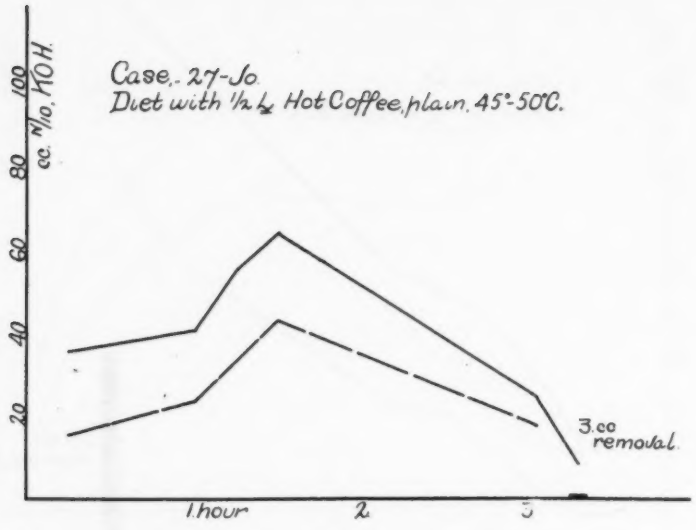
Case,- 27-Jo
Diet with 1/2 L. Hot Coffee, plain. 45°-50°C.
cc. N/10, HOH
100
80
60
40
20
3.cc removal.
1.hour
2
3

FIG. 27

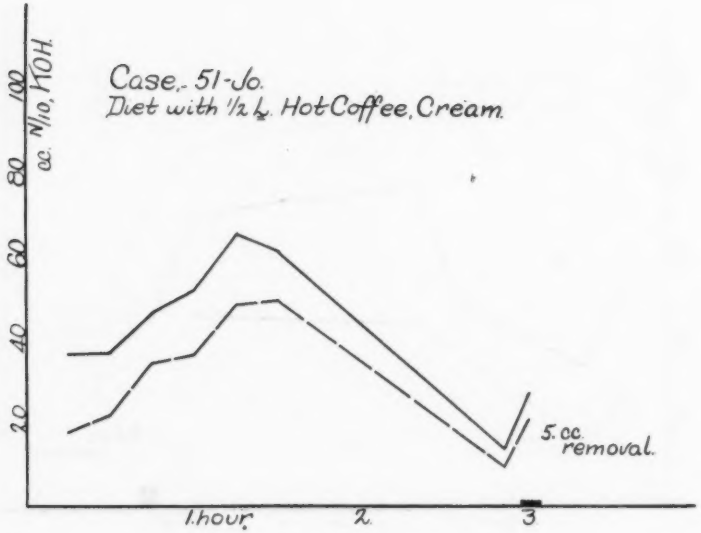
Case,- 51-Jo.
Diet with 1/2 L. Hot Coffee, Cream.
cc. N/10, HOH
100
80
60
40
20
5. cc. removal.
1.hour
2
3

FIG. 28

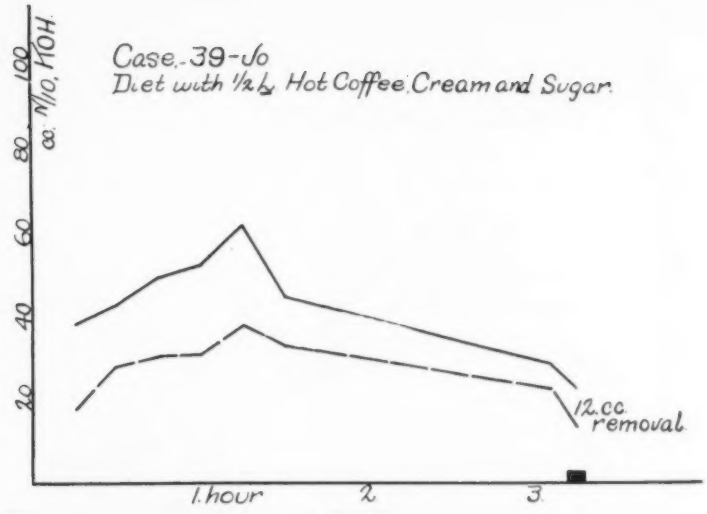
Case.-39-Jo
Diet with 1/2 L. Hot Coffee, Cream and Sugar.
cc. N/10. KOH
100
80
60
40
20
12 cc. removal
1 hour
2
3

FIG. 29

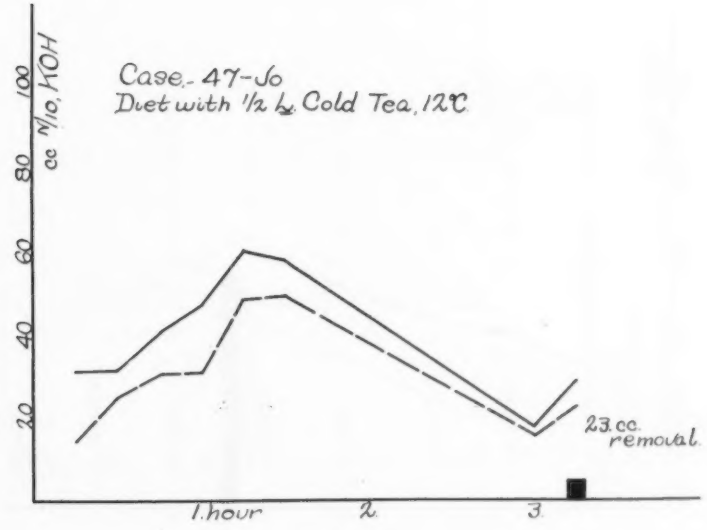
Case.-47-Jo
Diet with 1/2 L. Cold Tea, 12C
cc N/10. KOH
100
80
60
40
20
23 cc. removal
1 hour
2
3

FIG. 30

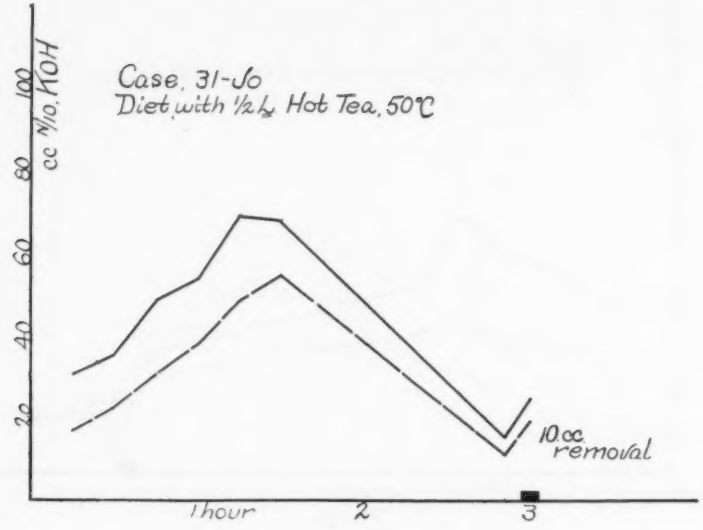
Case. 31-Jo
Diet with ½ L. Hot Tea, 50°C
cc N/10. KOH
100
80
60
40
20
10 cc removal
1 hour
2
3

FIG. 31

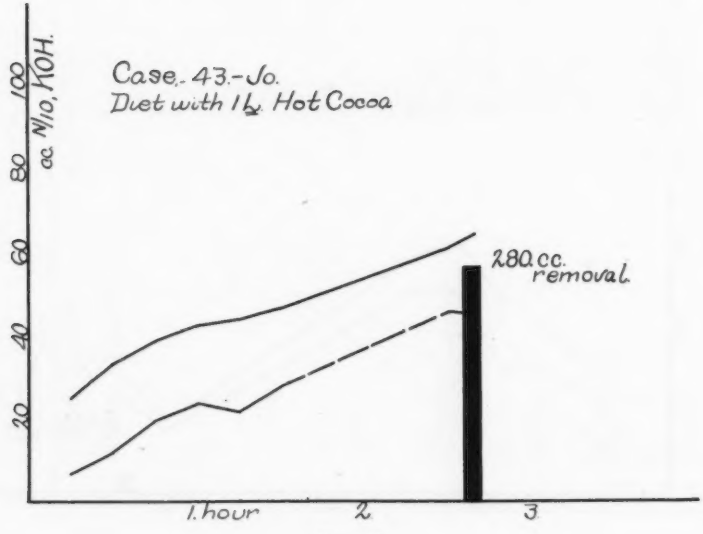
Case. 43.-Jo.
Diet with 1 L. Hot Cocoa
cc N/10, KOH.
100
80
60
40
20
280 cc. removal
1 hour
2
3

FIG. 32

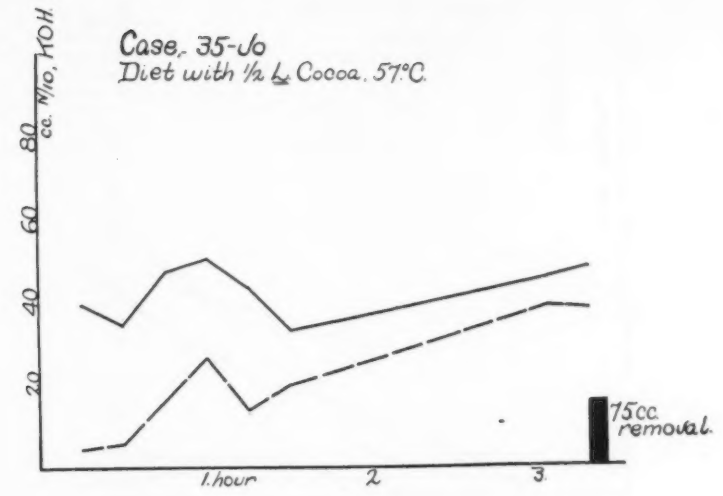
Case, 35-Jo
Diet with ½ L. Cocoa. 57°C.
cc. N/10, KOH
80
60
40
20
75cc. removal.
1. hour
2
3

FIG. 33

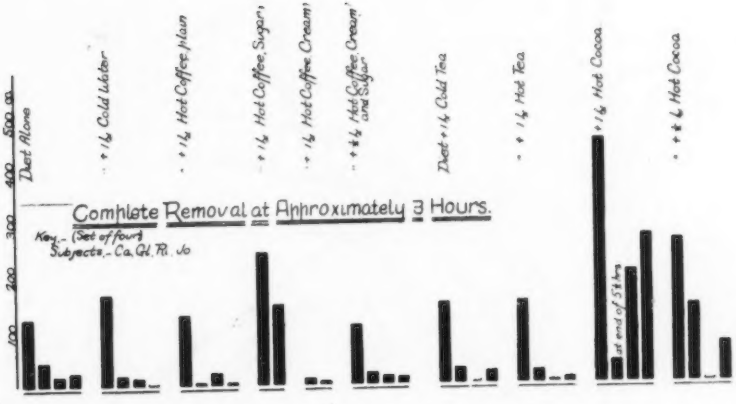
Diet Alone
+ ½ Cold Water
+ ½ Hot Coffee, Plain
+ ½ Hot Coffee, Sugar
+ ½ Hot Coffee, Cream
+ ½ Hot Coffee, Cream and Sugar
Diet + ½ Cold Tea
+ ½ Hot Tea
+ ½ Hot Cocoa
+ ½ L. Hot Cocoa
Complete Removal at Approximately 3 Hours.
Key – (Set of four) Subjects – Ca, Gl, Pt, Jo
100
200
300
400
500 cc.
at end of 5½ hrs.

FIG. 34

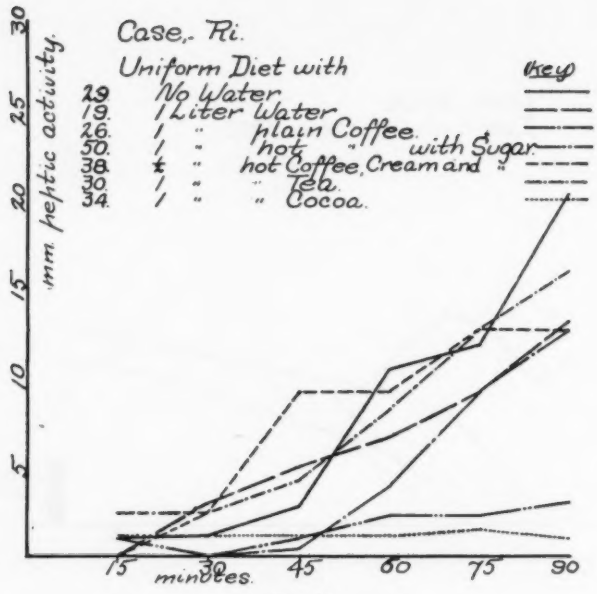
Case. Ri.
Uniform Diet with
29 No Water
19 1 Liter Water
26 1 " plain Coffee
50 1 " hot " with Sugar
38 ½ " hot Coffee, Cream and "
30 1 " " Tea
34 1 " " Cocoa
Key
mm. peptic activity.
5
10
15
20
25
30
15
30
45
60
75
90
minutes.

FIG. 35

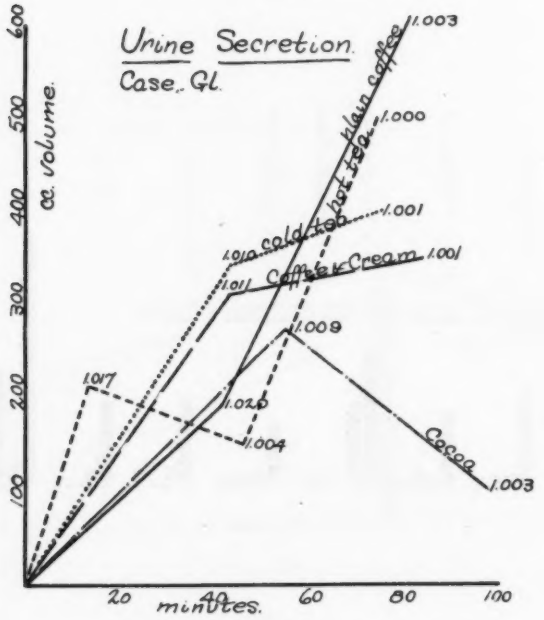
Urine Secretion
Case. Gl.
cc. volume
100
200
300
400
500
600
20
40
60
80
100
minutes.
1.003
plain coffee
1.000
hot Tea
1.001
1.010 cold tea
1.001
1.011 Coffee & Cream
1.009
1.017
1.020
1.004
Cocoa
1.003

FIG. 36

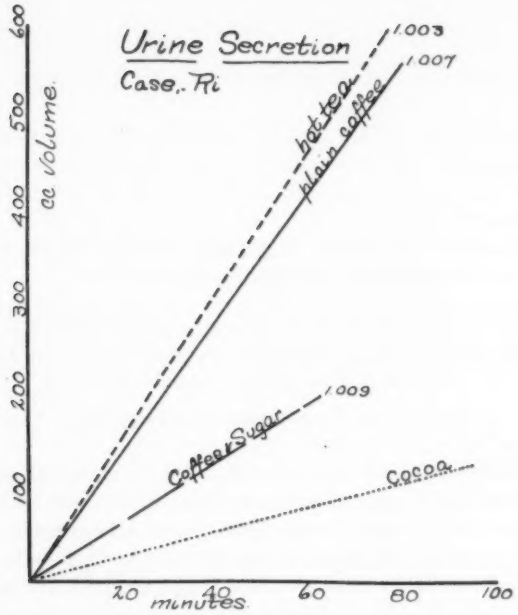
Urine Secretion
Case. Ri
cc volume
600
500
400
300
200
100
hot tea
plain coffee
coffee Sugar
Cocoa
1.003
1.007
1.009
20
40
60
80
100
minutes

FIG. 37

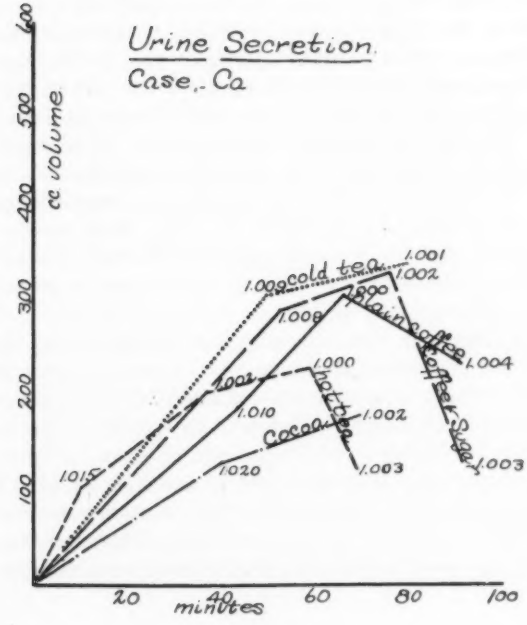
Urine Secretion
Case. Ca
cc volume
600
500
400
300
200
100
cold tea
plain coffee
hot tea
Cocoa
coffee Sugar
1.001
1.002
1.000
1.008
1.004
1.000
1.002
1.010
1.002
1.015
1.020
1.003
1.003
20
40
60
80
100
minutes

FIG. 38

I. RAPID BLOOD PLASMA PROTEIN DEPLETION AND THE CURVE OF REGENERATION

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The published work of Kerr, Hurwitz and Whipple (1) brings out several facts about the blood serum proteins which may be mentioned briefly before we go on to a consideration of the experiments given below. The *stability of the serum protein concentration* is truly remarkable and obviously of some importance to the body physiology. The normal level is quite constant and considerable deviations from this normal base line are not well tolerated by the body; in fact, profound shock may result. When the serum proteins are depleted or washed out by the technique employed the repair or regeneration of these proteins is a slow process requiring from 5 to 10 days, depending upon the amount removed and other factors. It is as difficult to reconstruct these proteins as it is for the body to repair and replace liver cells following an extensive liver injury. It appears that the liver is especially concerned in the normal regeneration of blood serum proteins. Fasting does not prevent serum protein regeneration, therefore it is possible for the body to release these substances or to construct serum proteins from its own protein end products. There is no evidence that increased nitrogen breakdown is responsible for this regeneration of serum protein.

The experiments of Kerr, Hurwitz and Whipple were different as to method of blood serum depletion when compared with the experiments given below. These earlier experiments were mostly done by *interval bleedings followed* in each instance by a return of the washed red corpuscles suspended in modified Locke's solution. Under such circumstances a dog was bled 100 to 200 cc. and after washing by centrifugalization the same red cells were returned intravenously in Locke's solution. This procedure was repeated several times during the day until the serum protein depletion was carried to a minimum figure.

This method has been used by Abel, Rowntree and Turner (2) and called "plasmapharesis." It is obvious that this experimental procedure introduced wide fluctuations in blood volume and it was suspected that the shock which resulted was to be explained by the repeated hemorrhages and infusions.

We shall use the term *plasma depletion* or *plasmapharesis* to indicate a removal of plasma proteins by means of repeated hemorrhage followed *or accompanied* by the replacement of like amounts of red cells suspended in a protein-free fluid. The plasma depletion in our experiments was effected by a method first introduced by Morawitz (3); bleeding and the removal of whole blood was simultaneous with the replacement of the red cell Locke's solution mixture The inflow and outflow volume was at all times constant and obviated any fluctuation in blood volume. All aseptic precautions were taken in manipulation, washing and final preparation of the red cell mixtures. The red cell mixtures were introduced at body temperature and the dog was kept warm during the experiment.

The method employed in these experiments enables an investigator to reduce the blood plasma proteins from the normal level of 5 to 6 per cent to a very low level of 1.5 to 0.9 per cent. This can be done in a matter of minutes (2 to 10 minutes) leaving the animal uninfluenced by the large and numerous fluctuations in blood volume and oxygen-carrying capacity of the blood which undoubtedly occur in the method used by Kerr, Hurwitz and Whipple. In addition it facilitates observations of that portion of the curve of protein regeneration immediately following a large single depletion and permits observations on an uninterrupted regeneration curve.

METHOD

The animals used were sound young dogs maintained on a mixed diet. In most cases no food was given the animal for a period of 12 hours preceding the experiment. Free access to water obtained. Under complete ether anesthesia and with aseptic precautions an incision was made either into the region of the femoral vessels or of the large vessels of the neck. The artery and vein were exposed and clamped. Into each was introduced a vaseline-coated cannula pointing toward the heart. Plasma removal was effected by withdrawing through the cannula placed in the artery large quantities of blood. This blood was allowed to flow into a graduated bottle. Simultaneously, a suspension

of washed corpuscles warmed to body temperature was injected under pressure through the venous cannula. This suspension was delivered from a flask which was also graduated. The graduations were used to permit a comparison of the inflow with outflow to be made at any time during the exchange. In this way inflow and outflow were observed and kept equal at all times. In order to maintain an even suspension of the injection mass, the latter was frequently shaken.

The fluid injected consisted of washed dog corpuscles suspended in a modified Locke's solution in the ratio of three parts packed corpuscles to two parts by volume of the saline mixture. The composition of the Locke's solution was: sodium chloride, 0.9 per cent; potassium chloride, 0.042 per cent; sodium bicarbonate, 0.02 per cent. The corpuscles in all cases were obtained from the blood of healthy dogs. This blood was drawn into sodium oxalate, centrifugalized, and the plasma removed from the sedimented corpuscles. The corpuscles were then washed twice in the modified Locke's solution by resuspension and centrifugalization. Aseptic precautions were observed in all these manipulations.

The exchange was effected in a period ranging in different animals from 2 to 25 minutes. The amount of fluid withdrawn varied in individual experiments from 60 per cent to 195 per cent of the animal's blood volume. The amount of blood simultaneously injected corresponded within a few cubic centimeters to the amount withdrawn. The blood volume was estimated as 10 cc. per 100 grams of body weight. In a number of experiments the actual blood volume was kindly determined for us by Dr. C. W. Hooper, using a dye method recently described. This paper (4) shows that the blood volume of active normal dogs as determined by the dye method is approximately 10 cc. per 100 grams of body weight.

At the end of the operative procedure the cannulae were withdrawn and the vessels ligated. Vaseline was applied liberally to the wound. In a few instances it was necessary to make a single suture through the subcutaneous tissues at the site of operation. In practically every experiment the wound healed quickly with little or no suppuration.

Samples of blood were collected through the arterial cannula at the beginning and at the end of the exchange and again 15 minutes later. Subsequent samples were taken from the jugular vein by means of a needle and syringe. On each of these occasions two samples were withdrawn. One of these was drawn into a 15 cc. hematocrit tube containing 3 cc. of 1 per cent sodium oxalate. The other sample was drawn into a plain heavy-walled glass test tube and allowed to clot. Both

of these samples were then centrifugalized at a high rate of speed. The serum from the clotted sample was then used for estimation of serum albumins, serum globulins and the non-protein fraction by the refractometric method of Robertson (5). Percentage corpuscles readings were made from the oxalated sample, correction being made for the amount of oxalate solution present. The plasma from this sample was also used for the determination of fibrin. This was carried out according to the method of Cullen and Van Slyke (6). This method consists in diluting 5 cc. of plasma in 100 cc. of salt solution. To this mixture 1.5 cc. of a 2.4 per cent solution of $CaCl_2$ was added to supply calcium and to promote clotting, and a Kjeldahl done on the mass of fibrin obtained.

The clinical condition of the animal was closely observed. The rectal temperature, rate of respiration, pulse, diarrhea and vomitus, as well as the general appearance of the animal, were noted. In cases in which death resulted, careful autopsies were performed at once. The clinical condition of the animal will be made the subject of a subsequent paper with a discussion of the peculiar type of shock which may develop under these conditions.

EXPERIMENTAL OBSERVATIONS

This paper in general deals with the recovery experiments but in certain tables we include many of the lethal shock experiments (tables 12, 13 and 14). For the sake of comparison we give in table 1 a type experiment which was followed promptly by fatal shock. Many of these experiments will be found in the next paper of this series and in that place the general discussion of this peculiar shock will be presented. It will be noted in table 1 that the reduction of total proteins, albumin and globulin is pretty uniform and is a fall to approximately one-third of normal. The emergency increase of protein during the 15 minutes following the plasma depletion is not as marked as usual (see table 2). There is no further increment of serum protein in the hour following this 15-minute sample and this may be explained in part by the profound shock. The content of red cells in whole blood as shown by the hematocrit is lower than usual and the fall which appears immediately after plasmapharesis would indicate the use of a red cell mixture containing fewer red cells than intended. This factor does not complicate the remaining experiments and we believe has no significance.

Experiment 104. (See table 1). 122 per cent exchange.

Dog 18–48. Young female bull dog. Weight 16.9 pounds. Blood volume on November 20, 1917 (by dye method) was 941 cc.

November 20, 1917. Under ether anesthesia 940 cc. blood were withdrawn from the left carotid artery. Simultaneously 1000 cc. of blood corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 10.5 minutes. Animal showed almost immediately a great fall in pulse pressure and arterial tension. Profound depression with forced irregular respiration developed in about an hour. Death 2 hours after the exchange. The autopsy findings are uniform in all fatal experiments and will be described in detail in the following paper.

TABLE 1

122 per cent blood volume exchange; dog 18–48; experiment 104

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange.....	5.7	4.2	1.5	2.0	0.25	47	
Immediately after...	1.9	1.4	0.5	1.6	0.12	35	Considerable hemolysis
15 minutes after.....	2.3	1.9	0.4	1.7	0.11	38	Considerable hemolysis
1 hour and 20 minutes.....	2.4	2.0	0.4	2.1		30	Fatal shock

Table 2 gives the results of an experiment which contrasts with this lethal shock experiment (table 1). The second experiment presents an even larger blood exchange in plasmapharesis but this dog is not disturbed by the procedure. It will be noted that there are marked individual differences in dogs as to their tolerance to this plasma depletion. Any given dog will show a considerable uniformity of reaction to a unit exchange but must be standardized to ascertain this reaction. Table 2 also shows a fall of total protein, albumin and globulin to about one-third normal following the plasmapharesis. We wish to call attention to the emergency increase in blood proteins which appears *within 15 minutes.* This is a characteristic reaction which obtains in practically all experiments (tables 11 and 12). The increase in serum proteins during the next 24 hours is very marked and exceeds 1 per cent protein—equivalent to more than 20 per cent of the total protein replaced in the blood serum. Subsequent regeneration of protein in the serum is slow and requires several days for complete recovery.

Experiment 69. (See table 2). 170 per cent exchange.

Dog 18–9. Young female bull dog. Weight 14 pounds. Blood volume on July 19, 1917 (by dye method) was 772 cc.

August 2, 1917. Under ether anesthesia 1081 cc. blood were withdrawn from the right femoral artery. Simultaneously 1081 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of exchange was 12 minutes. Following the exchange the temperature fell about 2 degrees, but returned to the original level within 2 hours. Pulse and respiration were fair at all times.

A much smaller volume exchange is shown in table 3, yet considerable shock resulted. It will be seen that the level of total proteins, albumin and globulin falls to approximately one-half of normal corresponding to the smaller exchange volume (90 per cent). There is a

TABLE 2

170 per cent blood volume exchange; dog 18–9; experiment 69

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	5.6	3.8	1.8	1.7	
Immediately after.....	2.0	1.3	0.7	1.4	Moderate hemolysis
15 minutes after.....	2.9	2.0	0.9	1.3	Moderate hemolysis. No shock
2d day.....	4.2	3.1	1.1	1.9	Slight hemolysis. Normal
3d day.....	4.5	2.5	2.0	2.1	
4th day.....	4.8	3.2	1.6	1.7	

moderate increase in serum proteins during the 15-minute period and less than usual during the first 24 hours following plasmapharesis. We cannot explain satisfactorily the remarkable drop in red cell hematocrit which is present after 11 hours and persists many days. Possibly the red cells used for infusion in this experiment had been seriously injured and went to pieces in the circulation. In confirmation of this suggestion we note the presence of hemolysis in blood samples taken on the first four days following the experiment. It may be suspected that an hemolysin was present in this dog's blood but there is reasonable doubt whether hemolysins actually do occur in the dog in sufficient amount to destroy large numbers of homologous red cells.

Experiment 103. (See table 3). 91 per cent exchange.

Dog 18–66. Young female bull mongrel. Weight 17 pounds.

November 15, 1919. Under ether anesthesia 700 cc. blood were withdrawn from the right femoral artery. Simultaneously 700 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of exchange was 9 minutes. Following the exchange the temperature showed little or no alteration from the original level. The pulse was regular but poor in tension for a number of hours. One-half cubic centimeter of adrenalin subcutaneously was given 4 hours after the operation. After 24 hours the animal was in excellent condition.

In all these experiments the washing out of plasma proteins is accomplished by a *rapid exchange.* The bleeding and simultaneous infusion of the red cell mixture occupies only a few minutes, the limits being

TABLE 3

91 per cent blood volume exchange; dog 18-66; experiment 103

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange.....	6.2	4.5	1.7	2.0	0.42	49	
Immediately after...	3.2	2.2	1.0	1.5		49	
15 minutes after.....	3.8	2.9	0.9	1.6	0.21	56	
3 hours.....	4.3	3.0	1.3	1.7	0.58	50	Moderate shock
11 hours.....	4.0	2.9	1.1	2.3	0.47	33	
2d day.....	4.3	2.8	1.5	2.6	0.48	35	Dog nomal. Hemolysis
3d day.....	4.8	3.7	1.1	2.5	0.47	29	Hemolysis
5th day.....	4.5	3.1	1.4	3.0	0.41	27	Hemolysis
6th day.....	5.3	3.9	1.4	2.3			
8th day.....	5.4	3.8	1.6	2.9	0.45	32	
10th day.....	5.7	4.1	1.6	2.0	0.42	32	
12th day.....	5.4	3.9	1.5	2.1	0.56	32	

2 to 25 minutes. Within these limits the speed of exchange, whether 2 minutes or 25 minutes, seems to make little difference. To make this point clear we may contrast tables 4 and 5. The first of these two experiments done on the same animal (table 4) shows the reaction following an exchange of 100 per cent done in 14 minutes. There was definite shock but a rapid recovery. The second experiment done on this dog after an interval of 2 weeks to insure complete recovery, shows the reaction following a very rapid 100 per cent exchange which was completed *within 2 minutes.* There was if anything less shock on this occasion than after the first exchange. It is interesting to note how closely

the curves of total protein, albumin and globulin in the two experiments coincide. The prompt rise in the 15-minute interval is identical and the initial fall corresponds to the other experiments discussed.

Experiment 93. (See table 4). 99 per cent exchange.

Dog 18–48. Young female bull dog. Weight 13.3 pounds.

October 4, 1917. Under ether anesthesia 600 cc. blood were withdrawn from the right femoral artery. Simultaneously 700 cc. of blood corpuscle suspension

TABLE 4

99 per cent blood volume exchange; dog 18–48; experiment 93

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	4.5	3.2	1.3	2.2	0.26	58	
Immediately after...	2.0	1.3	0.7	1.6	0.16	58	
15 minutes after.....	3.3	2.6	0.7	1.5	0.15	58	Definite shock
9½ hours.....	3.9	3.0	0.9	1.8	0.22	46	
2d day.....					0.18	54	Normal
3d day.....	4.0	3.1	0.9	1.9	0.30	42	
4th day.....	4.4	3.3	1.1	1.7	0.39		

TABLE 5

104 per cent blood volume exchange; dog 18–48; experiment 96

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.4	3.5	2.9	1.7	
Immediately after.....	2.1	1.3	0.8	1.3	
15 minutes after.....	3.2	2.2	1.0	1.6	Slight shock
10 hours.....	3.9	2.7	1.2	1.6	
2d day.....	4.5	2.6	1.9	1.9	Normal

were injected into the right femoral vein. The duration of exchange was 14 minutes. Animal showed definite signs of intoxication after about an hour following the exchange, with some bloody feces after about 5½ hours. After 24 hours animal appeared to have recovered completely.

Experiment 96. (See table 5). 104 per cent exchange.

Dog 18–48. Young female bull dog. Weight 13.8 pounds. "Plasmapharesis," 99 per cent exchange with a duration of 14 minutes, done on October 4 (exper. 93, table 4). Showed definite signs of intoxication.

October 18, 1917. Under ether anesthesia 650 cc. blood were withdrawn from the left femoral artery. Simultaneously 650 cc. of blood corpuscle suspension were injected into the left femoral vein. *The duration of exchange was 2 minutes.* Immediately following the exchange there was a slight transient fall in temperature. There was no immediate alteration of the pulse; however after about an hour the pulse was of poor volume and the animal appeared decidedly dull. Bloody feces were noted at this time. After 24 hours the animal appeared to be in good condition.

Table 6 gives valuable data concerning the speed of exchange in its relation to shock and the curve of protein regeneration. In this experiment the blood volume exchange of 75 per cent was *completed in 2.5 minutes.* There was no shock and we may compare the previous exchanges done on this same animal (Sept. 12, 1917, exper. 84, 80 per

TABLE 6

75 per cent blood volume exchange; dog 18–35; experiment 94

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.4	3.8	1.6	1.9	0.21	40	
Immediately after.....	3.0	2.2	0.8	1.5	0.19	48	
15 minutes after.....	3.7	2.7	1.0	1.8	0.23	54	
3¼ hours.....	4.6	3.4	1.2	1.8	0.26	61	No shock
6¾ hours.....	4.7	3.5	1.2	1.8	0.30	44	
9½ hours.....	4.1	2.9	1.2	1.5			Normal
2d day.....	4.9	3.6	1.3	1.9	0.44		
3d day.....	4.4	3.1	1.3	2.8			
4th day.....	4.6	2.4	2.2	2.4			

cent exchange, shock very slight, time 7 minutes; October 3, 1917, exper. 92, 74 per cent exchange, no shock, time 14 minutes). The curve of serum protein depletion and regeneration is similar to other experiments. The small per cent exchange lowers the total protein to 3.0 per cent and the emergency increase is definite within 15 minutes, giving a rise to 3.7 per cent. It appears that the emergency reaction by which a considerable amount of serum protein is thrown into the blood stream, can be called out by a large or small exchange using this method.

Experiment 94. (See table 6). 75 per cent exchange.

Dog 18–35. Young female bull dog. Weight 16 pounds. "Plasmapharesis," 80 per cent exchange, done in 7 minutes on September 12, 1917, showing very

slight shock (exper. 84); another 14 per cent exchange done in 14 minutes on October 3, 1917, showing no shock (exper. 92). Blood volume on October 9, 1917 (by dye method) was 671 cc.

October 11, 1917. Under ether anesthesia 545 cc. blood were withdrawn from the right carotid artery. Simultaneously 545 cc. of blood corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 2½ minutes. Animal showed a transient drop in temperature of 3 degrees mmediately following the exchange. Animal showed little or no signs of ntoxication.

Table 7 shows a remarkably prompt return to normal after a large exchange (109 per cent). The total proteins fell to a level of 50 per cent normal which is a normal reaction. We cannot explain the figures, which appear to show a peculiar reaction on the part of the albumin and globulin fractions. These peculiar reactions will appear at

TABLE 7

109 per cent blood volume exchange; dog 18–20; experiment 89

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.0	3.6	1.4	1.9	0.35	50	
Immediately after...	2.5	0.9	1.6	1.4	0.12	60	
15 minutes after.....	3.0	1.5	1.5	1.6	0.11	73	
4½ hours.....	4.2	2.2	2.0	1.7	0.13	73	Very slight shock
2d day.....	5.1	2.9	2.2	2.1		41	Normal

times in spite of every care used in the method, but we are inclined to suspect technical errors as in part responsible. The return of the total protein to normal within 24 hours is unusual and would indicate an unusually large emergency reserve. The peculiar rise in red cell hematocrit will be found in this experiment and in a few subsequent experiments. That it appears in the 15-minute and 4-hour samples but not in the sample taken immediately after the exchange is very perplexing. There is no severe shock to account for any withdrawal of fluid from the blood. We have no convincing explanation to offer.

Experiment 89. (See table 7). 109 per cent exchange.

Dog 18–20. Young female bull dog. Weight 16.1 pounds.

September 20, 1917. Under ether anesthesia 800 cc. blood were withdrawn from the femoral artery. Simultaneously 800 cc. of blood corpuscle suspension were injected into the femoral vein. The duration of the exchange was 14½

minutes. Animal showed little or no depression. The temperature fell 3 degrees, however, and the animal shivered considerably for 8 or 9 hours. In good condition after 24 hours.

Table 8 shows another typical experiment giving the usual curve of blood proteins following plasmapharesis of moderate amount (90 per cent). The *fibrin curve* is given in this experiment and we believe this illustrates the usual reaction on the part of this plasma globulin. The method used gives certain opportunities of error when small amounts of plasma are analyzed for fibrin. More work in this field has been completed by Mr. Foster in this laboratory and will soon be published. We do not wish to put too much emphasis on these figures.

TABLE 8

90 per cent blood volume exchange; dog 18–68; experiment 105

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	5.5	4.0	1.5	2.2	0.42	
Immediately after	2.8	2.1	0.7	1.7	0.22	
15 minutes after	3.5	2.8	0.7	1.8	0.27	
2½ hours	4.6	3.3	1.3	1.8	0.30	Moderate shock
5 hours					0.19	
2d day	4.8	3.8	1.0	2.0	0.56	Normal
4th day	4.9	3.4	1.5	1.3	0.42	
6th day	5.4	3.7	1.7	2.1	0.49	

Experiment 105. (See table 8). 90 per cent exchange.

Dog 18–68. Young female bull dog. Weight 15.3 pounds.

November 21, 1917. Under ether anesthesia 623 cc. of blood were withdrawn from the right femoral artery. Simultaneously 623 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 9 minutes. The animal showed moderate depression and slight decrease in pulse volume for several hours.

Tables 9 and 10 give figures to show the low level of serum proteins which may be effected by very large blood volume exchanges (159 and 195 per cent). The usual normal dog will not tolerate such large exchanges without exhibiting profound and often fatal shock. These two dogs were unusually resistant to this experimental procedure and give us the opportunity to study the reaction following such large

exchanges uncomplicated by shock or notable hemolysis. The low level of total proteins is to be expected and one experiment (table 10) reaches the minimum figure for total protein (0.9 per cent). We have no observation in any of our experiments to show a lower level of protein in the blood stream. The protein regeneration is very rapid in the 15-minute period as well as in the following 24 hours, indicating considerable emergency reserve material.

TABLE 9

159 per cent blood volume exchange; dog 17–215; experiment 67

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.2	3.6	2.6	1.7	
Immediately after.....	1.3	0.3	1.0	1.6	Slight hemolysis
15 minutes after.....	2.2	1.2	1.0	1.5	Slight hemolysis. Slight shock
2d day.....	4.1	2.7	1.4	1.4	Normal
3d day.....	4.3	2.1	2.2	2.0	
4th day.....	5.2	2.9	2.3	1.7	

TABLE 10

195 per cent blood volume exchange; dog 17–232; experiment 70

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.3	3.7	1.6	1.9	
Immediately after....	0.9	0.3	0.6	1.5	
15 minutes after.....	1.4	0.7	0.7	1.7	Very slight shock
2d day.....	4.3	2.3	2.0	1.5	Normal
3d day.....	5.5	3.8	1.7	1.9	

Experiment 67. (See table 9). 159 per cent exchange.

Dog 17–215. Adult female fox terrier. Weight 15.25 pounds. Blood volume on July 19, 1917 (by dye method) was 858 cc.

July 31, 1917. Under ether anesthesia 1105 cc. of blood were withdrawn from the right femoral artery. Simultaneously 1105 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 10 minutes. There was slight decrease in force of pulse beat for about an hour. Animal showed little sign of depression thereafter.

Experiment 70. (See table 10). 195 per cent exchange.

Dog 17–232. Young female coach dog. Weight 13.5 pounds. Blood volume on July 19, 1917 (by dye method) was 714 cc.

August 3, 1917. Under ether anesthesia 1200 cc. blood were withdrawn from the right femoral artery. Simultaneously 1200 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 10 minutes. The pulse pressure was poor for about an hour following the exchange.

TABLE 11

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	TOTAL PROTEIN				TOTAL DROP IN PROTEIN	PER CENT PROTEIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of exchange	15 minutes after exchange	Second day		In 15 minutes	In 24 hours		Before exchange	At end of exchange	15 minutes after exchange	Second day
67	60	5.3	2.8	3.9		2.5	1.1		0				
74	92	5.5	3.7	3.8		1.8	0.1		0	50	58	58	
74	87	6.2	3.3	3.9		2.9	0.6		+++	58	58	55	
75	94	5.4	3.0	3.7	4.9	2.4	0.7	1.9	0	40	54	54	
75	90	5.3	2.9	3.4	4.3	2.4	0.5	1.4	++	48	54	55	38
80	84	5.6	3.1	3.1	6.6	2.5	0.0	3.5	+	50	63	58	33
80	82	5.6	2.4		4.4	3.2		2.0	0		50		44
84	74	4.9	2.6			2.3			*				
89	101	5.5	2.1	2.4		3.4	0.2		+++	60	41	53	
90	105	5.5	2.8	3.5	4.8	2.7	0.7	2.0	++	43	49	52	
91	103	6.2	3.2	3.8	4.3	3.0	0.6	1.1	++	49	49	56	35
94	62	4.9	1.8	2.8	4.8	3.1	1.0	3.0	++				
96	61	5.5	2.7	3.6		2.8	0.9		0				
98	64	6.3	3.0	2.8		3.3	−0.2		+				
99	81	4.5	1.2	2.5	4.1	3.3	1.3	2.9	++				
99	93	4.5	2.0	3.3	4.0	2.5	1.3	2.0	+	58	58	58	42
100	100	5.6	3.0	2.9	4.6	2.6	−0.1	1.6	+	46	62	64	36
Averages		5.4	2.7	3.3	4.8	2.7	0.6	2.1		50	55	56	38

Shock readings: + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

* Death from overdose of ether.

A summary of certain factors in many plasma depletion experiments will be found in tables 11 and 12 and the *average figures* give much interesting information. The averages of the experiments which show 100 per cent or less of blood volume exchange (table 11) show an identical emergency increase in the blood serum proteins. The two tables are practically in accord and we note that the average replacement of serum protein during the 15 minutes following the plasmapharesis

amounts to 0.5 to 0.7 per cent protein—which is an increase of 10 to 14 per cent of the total proteins. The increase during the 24 hours following the plasma depletion is considerable and amounts to 2.0 per cent protein which is an increase of 40 per cent total protein, figuring 5.0 protein per cent as the normal for a healthy dog.

Further analysis of the blood cell hematocrit figures is of interest. It is unfortunate that we did not obtain hematocrit readings in all our experiments. It is clear that the normal hematocrit before the ex-

TABLE 12

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	TOTAL PROTEIN				TOTAL DROP IN PROTEIN	PER CENT PROTEIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of exchange	15 minutes after exchange	Second day		In 15 minutes	In 24 hours		Before exchange	At end of exchange	15 minutes after exchange	Second day
102	80	5.6	2.6	2.8		3.0	0.2		+++				
104	96	6.4	2.1	3.2	4.5	4.3	1.1	2.4	+	52	66		38
108	98	6.3	2.2	2.7		4.1	0.5		+++	33	44	53	
109	89	5.0	2.5	3.0	5.1	2.5	0.5	2.6	+	50	60	73	41
110	83	5.8	2.3	2.8	4.2	3.5	0.5	1.9	+				46
118	63	7.1	4.2		4.8	2.9		−0.1	+				
122	104	5.7	1.9	2.3		3.8	0.4		+++				
131	66	5.7	1.0	3.1	4.0	4.1	1.5	2.4	++	47	35	38	30
141	68	5.5	2.6	2.5	3.9	2.9	−0.1	1.3	0				
159	67	6.2	1.3	2.2	4.1	4.9	0.9	2.8	+				
170	69	5.6	2.0	2.9	4.2	3.6	0.9	2.2	++				
191	77	5.9	0.9	1.2		5.0	0.3		+++				
195	70	5.3	0.9	1.4	4.3	4.4	0.5	3.4	0				
Averages		5.8	2.0	2.5	4.3	3.8	0.6	2.1		46	51	55	39

Shock readings: + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

periment is approximately 50, which is an indication that healthy dogs were used. There is a slight increase in the hematocrit figures at the end of the blood exchange but only to 55 per cent. This assures us that a suitable number of red cells was introduced in the red cell Locke's solution mixture which replaced the blood. There is a trifling increase in the average hematocrit figures for the 15-minute sample but only to 56 per cent, which may indicate a very slight blood concentration due to loss of Locke's solution from the circulation.

There is a distinct fall in hematocrit during the 24 hours following the plasmapharesis—an average of 7 to 12 per cent below the initial figure. This probably indicates a true loss of red cells as we must recall the fact that these red corpuscles which are introduced have

TABLE 13

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	FIBRIN IN PER CENT				TOTAL DROP IN FIBRIN	PER CENT FIBRIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of exchange	15 minutes after	Second day		In 15 minutes	In 24 hours		Before exchange	At end of exchange	15 minutes after exchange	Second day
74	92	0.21	0.16	0.15	0.28	0.05	−0.01	0.12	0	50	58	58	
74	87	0.18	0.08	0.15		0.10	0.07		+++	58	58	55	
75	94	0.21	0.19	0.23	0.44	0.02	0.03	0.25	0	40	48	54	
75	90	0.25	0.15	0.20	0.35	0.10	0.05	0.20	++	48	54	55	38
80	84	0.17	0.06	0.03	0.40	0.11	−0.03	0.34	+	50	63	58	33
80	82	0.17	0.08	0.13	0.33	0.09	0.05	0.25	0		50		44
84	74	0.36	0.15			0.21			*				
89	101	0.24	0.11	0.11		0.13	0.00		+++	60	41	53	
90	105	0.42	0.22	0.27	0.56	0.20	0.05	0.34	++	43	49	52	
91	103	0.42	0.19	0.21	0.48	0.23	0.02	0.29	++	49	49	56	35
99	93	0.26	0.16	0.15	0.30	0.10	−0.01	0.14	+	58	58	58	42
99	81	0.32		0.19					++				
100	100	0.25	0.16	0.16	0.45	0.09	0.00	0.29	+	46	62	64	36
102	80	0.42		0.26					+++				
104	96	0.50	0.13	0.14	0.40	0.37	0.01	0.27	+	52	66		38
108	98	0.25	0.12	0.11		0.13	−0.01		+++	33	44	53	
109	89	0.35	0.12	0.11		0.23	−0.01		+	50	60	73	41
110	83	0.75	0.18	0.30	0.44	0.57	0.12	0.26	+				46
122	104	0.50	0.13	0.14	0.40	0.37	0.01	0.27	+++	52	66		38
191	77	0.44	0.13	0.10		0.31	−0.03		+++				
Averages..		0.33	0.14	0.17	0.40	0.19	0.02	0.25		49	55	57	39

Shock readings: + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

* Death from overdose of ether.

been submitted to considerable manipulation in the necessary washing previous to the injection. Dogs' corpuscles too are notoriously fragile. We may assume for the present at any rate that many of the red cells which were introduced had been seriously injured and went to

pieces in the blood stream during the 24 hours following the blood exchange.

We can review the *fibrin analyses* in table 13 and at once a decided difference appears when we compare the serum protein curve with that of the plasma globulin, fibrinogen. The exchange of blood reduces the fibrin content to about the same level—that is, we can wash out the same percentage of fibrinogen by the usual plasmapharesis as we do in the case of the serum proteins. The fibrin content is reduced to a little less than one-half normal—from 0.33 to 0.14. During the 15 minutes following the plasma depletion there is no emergency reaction on the part of the fibrin as is so constant for the serum proteins. During the next 24 hours the fibrin is restored completely to normal. This may mean that there is no emergency reserve of the fibrin as it can be produced so rapidly in the body in any emergency. We know of many other facts which point to *complete dissociation* of fibrin and other blood proteins as to production and repair and general usefulness in the body economy.

DISCUSSION

A theoretical consideration of the factors involved in this protein replacement is difficult at this time. It may be claimed that this increase represents, in part at least, not a true increase in the quantity of circulating serum protein, but is the result of the escape from the circulation of fluid poor in protein material. However, if any considerable escape of fluid from the circulation were to occur, one would expect to note a rise in the percentage of red cells in the circulating medium. That no sufficient change in the cell-plasma ratio does occur can be seen from the hematocrit figures given in tables 11 and 12. On the other hand, it may be that the figures represent an actual influx of protein into the circulating medium. Such an influx could conceivably come from some tissue or organ which serves as a storehouse for this type of protein material. Seitz (7) thinks that the liver acts as such a storehouse. Earlier work in this laboratory by Kerr, Hurwitz and Whipple (1) shows a lack of reserve production of serum proteins after plasmapharesis in the Eck fistula dog. This indicates that liver insufficiency may impair the normal emergency reproduction of blood proteins.

Of particular interest is the rather remarkable increase in the 15 minutes immediately following the end of the experimental depletion.

This, if blood volume changes be excluded, appears to be truly a throwing in of ready-formed materials.

While the depletion curve of the fibrin fraction of the plasma proteins brought about by our experimental procedure compares closely with the curve of depletion of the serum proteins, still a distinctly different type of curve of fibrin repletion is revealed. A fairly typical experiment is presented in table 8. This point may also be studied by an examination of the results given in table 13. In these tables it may be seen that the rapid rise immediately following the procedure of depletion which is typical of the serum proteins is absent or at least negligible in the case of fibrin. However, the body seems to be able to supply large amounts of this protein in a space of 24 hours, for as the summary in table 13 shows, the fibrin on the day following the exchange is already as high as the original figure, or, as occurs in some cases, even higher. When such an over-production does occur the level usually returns to normal in one to two days.

This lack of correspondence between the regeneration figures for serum proteins and for fibrin protein in the period of initial regeneration, we believe furnishes additional evidence against a theory which would account for all changes in protein concentration in this period by the loss from the circulation of fluids poor in protein. For, in such a case, the concentration of the proteins might be expected to occur to practically the same degree in each. That this does not occur tends to strengthen the evidence given by the hematocrit figures.

It may be pointed out that the curve of serum protein regeneration is very different for this type of experiment when compared to the experiments of Kerr, Hurwitz and Whipple. We believe that these differences are to be explained wholly by the differences in the experimental depletion of the serum protein. Kerr, Hurwitz and Whipple used *interval depletions* of smaller amounts but repeated many times during a single day. In this manner they undoubtedly removed much of the large emergency reserve which is so conspicuous in the 24-hour regeneration in the experiments tabulated above. Therefore Kerr, Hurwitz and Whipple observed a curve of protein regeneration which was much more prolonged before a return to normal was observed. These experiments supplement the earlier ones and strengthen their conclusions.

SUMMARY

A rapid depletion of serum proteins is brought about in these experiments by the introduction of normal red blood cells suspended in a modified Locke's solution, care being taken to keep equivalent the volume of blood removed from the artery and the volume of red blood cell suspension simultaneously injected into the vein.

The serum protein depletion is roughly proportional to the size of this exchange and it is noteworthy that the rapid depletion of the total serum proteins can rarely be carried below 1.0 per cent without causing a fatal reaction.

An increase in serum protein concentration (serum protein replacement) begins immediately following the exchange or plasmapharesis. The increase is very rapid during the first 15 minutes following the exchange. The increase in serum proteins is more gradual thereafter during the first 24 hours and still more sluggish during the next few days. The normal level may be reached in 2 to 7 days.

The rapid replacement of serum proteins during the first 15 minutes following the exchange indicates some reserve supply of this material perhaps held in the body cells. The emergency supply is evidently small and the production of other similar material is difficult and requires time.

The blood fibrin reacts in a different fashion. The same initial fall is not followed by a rapid rise in the first 15 minutes. The recovery however is complete within 24 hours and probably earlier than this. Fibrin is a very labile protein as compared with the serum albumin and globulin.

Blood volume fluctuations are probably very little concerned in these experimental results. The red blood cell hematocrit ratio shows but little change during the period of initial reaction.

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II. SHOCK AS A MANIFESTATION OF TISSUE INJURY FOLLOWING RAPID PLASMA PROTEIN DEPLETION

The Stabilizing Value of Plasma Proteins

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In the preceding communication we have established the curve of serum protein regeneration following a single rapid replacement of whole blood with a red cell Locke's solution mixture. This plasma depletion (plasmapharesis) washes out more or less of the blood proteins and lowers the concentration of the blood proteins in the circulating blood. In the preceding article we have submitted many experiments which are associated with little or no "shock," using this term in the familiar clinical sense. In this paper we wish to discuss more particularly those cases which are associated with severe or lethal shock. A number of such experiments are given in detail below. This intoxication associated with plasmapharesis has been noted by the earlier workers: Morawitz (1), Abel, Rowntree and Turner (2) and Kerr, Hurwitz and Whipple (3). A variety of explanations has been given.

The *physiological value of the serum proteins* is admittedly little understood and we believe our experiments throw some light on this point. Published work from this laboratory (3) indicates that the serum proteins cannot be concerned with the nutrition of the body cells and the constant exchange between food protein and body protein. The experiments outlined below suggest rather strongly that one important function of these proteins is their "stabilizing value."

The *stabilizing value* of the blood serum proteins is brought out with especial emphasis by two experiments (tables 19 and 20). The dog is bled large amounts from the femoral artery while simultaneously equal amounts of a washed red cell, dialyzed serum mixture are injected into the femoral vein. No shock followed an exchange of

large size which would surely have been fatal if the dialyzed serum had been replaced by Locke's solution as in the standard plasmapharesis. During the dialysis of the serum it underwent considerable dilution while the dialyzable substances were being removed, but this dilute dialyzed serum was still able to protect the body cells against the shock which develops if the blood proteins are too much diluted as in the routine plasma depletion. We believe that this furnishes the last bit of evidence to show that the blood serum proteins make up an essential part of the environmental complex of the body cells. Too great a dilution of these substances invariably results in profound injury of certain cells and a reaction identical with "clinical shock."

When these protein substances are suddenly washed out of the blood serum there is a certain amount of similar material thrown in as an emergency reserve. If the depletion is too severe the body cells are injured by the very persistence of this abnormal condition and the condition of "shock" supervenes. Further it is evident that certain body cells are more sensitive than others to changes in the serum protein content—for example, liver cells. That these facts have some significance in relation to the general problem of clinical shock is at once evident.

Other experiments (3) already cited give proof that the simple plasma depletion with more or less clinical shock is associated with a certain amount of cell injury, as shown by the rise in urinary nitrogen in the two days following the exchange. There is neither gross nor histological evidence of cell necrosis, but this increase in nitrogen must come from body protein. This is further evidence for actual *cell injury as an essential part of the clinical complex named "shock."*

It may be noted also that when once the clinical picture of "shock" is established in these experiments we have been unable to save the animal by any of the familiar clinical measures, even by infusion of whole blood. The *essential injury* in these experiments is *cell protoplasm injury* induced by a sudden change in the colloidal solution which forms the normal environment of these cells. This may be a new type of cell injury but it may help us to understand the more complex cell injury which is probably responsible for "surgical shock."

EXPERIMENTAL OBSERVATIONS

The various experimental methods have been described in detail in the preceding communication. To save repetition we may refer to some of the experiments detailed in the first paper of this series. The experiments given below are only types which illustrate a characteristic reaction and usually represent groups of similar experiments.

The first two tabulated experiments (tables 14 and 15) illustrate the reaction which was so common in the experiments of paper I of this series. In addition these two experiments done on the same dog at an interval of three weeks show that this procedure (plasmapharesis) does not sensitize a dog to any subsequent repetition of this procedure. This shock so exactly resembles the anaphylactic shock in dogs that it seemed necessary to exclude this possibility. Other experiments giving the same negative results need not be instanced.

TABLE 14

80 per cent blood volume exchange; very slight shock; dog 18–35; experiment 84

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	5.6	4.6	1.0	2.4	0.17	50
Immediately after	3.1	2.5	0.6	2.8	0.06	63
15 minutes after	3.1	2.9	0.2	2.6	0.03	58
4 hours	4.1	3.3	0.8	2.5	0.06	58
8 hours	6.1	4.9	1.2	2.5	0.16	45
2nd day	6.6	5.5	1.1	2.7	0.40	33

The curve of protein regeneration during the eight hours following this plasma depletion is beautifully shown in both experiments. The emergency reserve was sufficient to replace all the serum proteins removed (table 14) but it is noted that the total drop in serum protein was but 2.5 per cent total protein.

Experiment 84. (See table 14). 80 per cent exchange.

Dog 18–35. Female bull pup. Weight 15 pounds. Appears to be in excellent condition.

September 12. Under ether anesthesia 545 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 545 cc. of Locke's corpuscle suspension were injected into the right femoral vein. The exchange was effected in 7 minutes. There was a fall in rectal temperature of about 1°C. following the exchange. No definite sign of intoxication was noted except for a slight amount of vomiting 2 hours following the exchange. The animal appeared to be in good condition on the 2nd day.

Experiment 92. (See table 15). 74 per cent exchange.

Dog 18–35. Female bull pup. Weight 16.25 pounds. On September 12 an 80 per cent exchange was effected in 7 minutes without any decided signs of intoxication (see table 14).

October 3. Dog seems to be in excellent condition. Under ether anesthesia 545 cc. of blood were withdrawn from the left femoral artery. Simultaneously and at the same rate 545 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The exchange was effected in 14 minutes. There was practically no alteration in rectal temperature and at no time were there any signs of intoxication.

Experiment 61. No clinical shock. 96 per cent exchange.

Dog 18–7. Female terrier pup. Weight 11 pounds. Estimated blood volume (by dye method) 530 cc.

July 19. Under ether anesthesia 480 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 480 cc. of Locke-corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 12 minutes. There was practically no disturbance in rectal temperature. There was a slight amount of drowsiness for several hours. Otherwise no disturbance was noted. The total serum proteins fell from the initial value of 5.5 per cent at the beginning of the exchange to a level of 2.7 per cent at the end of the exchange. Fifteen minutes later a value 3.6 per cent was found. No samples were taken subsequently. At no time was there any decided alteration in the albumin-globulin ratio.

TABLE 15

74 per cent blood volume exchange; no clinical shock; dog 18–35; experiment 92

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	5.5	4.0	1.5	1.8	0.21	50
Immediately after	3.7	2.7	1.0	1.7	0.16	58
15 minutes after	3.8	2.9	0.9	1.8	0.15	58
2½ hours	4.3	3.4	0.9	1.8	0.18	48
5½ hours	4.6	3.5	1.1	1.8	0.21	48
2nd day					0.28	

The next group of experiments illustrates the fatal shock which may develop following an exchange of blood equal to 100 per cent blood volume or more. From these and other experiments it is obvious that the body can supply an emergency reserve of serum proteins even during the period of profound shock which precedes death (2 to 5 hours). Moreover the ratio of albumin and globulin is not especially disturbed as is so frequently seen in severe intoxication due to bacterial invasion.

The clinical and anatomical pictures described in this condition of shock following plasma depletion are very constant and resemble in the dog the reaction observed in fatal anaphylaxis. The fall in blood pressure may be delayed several minutes—sometimes 30 minutes after completion of the exchange—but the fall in temperature is prompt. At times there may be a subsequent rise in temperature even in fatal intoxication, but often the loss of temperature control is complete and rectal temperatures of 30°C. may be recorded. Gastro-intestinal disturbance is the rule. Vomiting and diarrhea are seen early, sometimes within 30 minutes, and persist. This watery, blood-tinged diarrhea is common in fatal cases. Mucus may be very abundant in certain cases, even occasionally when recovery takes place following a severe intoxication. The dull lethargic appearance with clinical prostration is very typical of this type of shock. This picture corresponds closely with the surgical condition of "shock" associated with intoxication (for example, intestinal obstruction) or hemorrhages or prolonged operative manipulation.

The *autopsy findings* also are very uniform. For these the description of a single case will suffice. Blood removed from the heart at autopsy or from the veins at intervals before death may show delayed coagulation but this is not uniform. The fibrin content is low because this plasma protein like the serum proteins has been washed out by the exchange. The liver, spleen and kidneys show engorgement, usually most marked in spleen and liver. The thorax, heart and lungs are negative. The stomach may be pale or slightly injected. The entire small intestine shows congestion of its mucosa often more marked in the upper tract. The mucosa may be velvety, purplish red and coated with thick creamy mucous. All degrees of congestion are found. The lumen contains a thin, watery, blood-tinged fluid in which more or less mucus is present. The colon shows the same material and a mottled congested mucosa.

It will be noted that this picture of shock is almost identical with that produced by large doses of adrenalin, clamping of aorta or vena cava and trauma of the intestines, recently studied and described by Erlanger (5).

Experiment 98. (See table 16). 108 per cent exchange.

Dog 18–20. Female bull-terrier pup. Weight 19.4 pounds. On September 20 an exchange of 109 per cent in 14½ minutes produced moderately severe shock.

October 31. Under ether anesthesia 950 cc. of blood were withdrawn from the left femoral artery. Simultaneously and at about the same rate 1000 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of

the exchange was 12½ minutes. The rectal temperature fell about 1°C. during the exchange. Subsequently there was a fall of 1° more, when death occurred. The arterial tension was fairly good at the end of the exchange but became quite poor in the course of the next 15 minutes. It remained poor until death. Deep respiration developed in the course of the first hour following the exchange. No marked signs of depression or loss of power of attention appeared for about 2 hours after the exchange. The condition then became rapidly worse and death occurred 1 hour later.

Autopsy shows swollen congested spleen. The liver is deep red, the lobulation is obscure. The mucosa of the entire intestinal tract is congested. There is a considerable excess of mucus. The other organs are negative. Blood drawn from the heart at time of autopsy when placed in a test tube clots in 25 minutes; that which is left in contact with the tissues clots in 10 minutes. The clot formed is quite flabby.

Experiment 101. (See table 17). 89 per cent exchange.

Dog 18–5. Young male terrier. Weight 18.5 pounds. On July 18 an exchange of 67 per cent produced a very mild grade of shock.

TABLE 16

108 per cent blood volume exchange; fatal shock; dog 18–20; experiment 98

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange	6.3	4.4	1.9	2.1	0.25	33	
Immediately after	2.2	1.3	0.9	1.4	0.12	44	
15 minutes after	2.7	1.7	1.0	1.6	0.11	53	
2 hours	3.6	2.5	1.1	2.1	0.12	42	Death

November 8. Under ether anesthesia 750 cc. of blood were withdrawn from the left femoral artery. Simultaneously 750 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 11 minutes. The rectal temperature fell 2°C. as a result of the exchange but returned subsequently to the original level of slightly above 40°. The *arterial tension* was good at the end of the exchange but became poor within the course of 30 minutes. Definite signs of general depression or "shock" appeared within an hour following the end of the exchange. The power of attention was completely lost 2 hours later. Death occurred 5 hours after the exchange.

Autopsy: The thymus is somewhat larger than normal. Otherwise the thoracic organs are negative. The spleen is moderately enlarged and congested. The liver is negative except for pronounced indistinctness of lobulation. The mucosa of the duodenum is slightly reddened, but no excess of mucus is found in the lumen.

Experiment 76. Fatal shock. 178 per cent exchange.

Dog 18–7. Female terrier pup. Weight 10.5 pounds.

A 96 per cent exchange was carried out on July 19 (see exper. 61) with practically no sign of shock.

On July 26 an exchange of 118 per cent was effected with very slight reaction. Immediately following this second exchange an injection of phosphorus was given. No definite injury was noted (see exper. 65, table 24).

August 16. Animal appeared to be in excellent condition. Under ether anesthesia 850 cc. of blood were withdrawn from the right carotid artery. Simultaneously and at about the same rate 900 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 7 minutes. Within a few minutes definite signs of shock appeared. The pulse rapidly diminished in volume, the respiration became irregular and the rectal temperature fell steadily from the original of 38.5°C. to 36.1° at the time of death, 1¼ hours following the exchange.

Autopsy: The thoracic organs are negative. The spleen, liver and kidneys show moderate congestion. The upper part of the small intestines shows marked thickening and congestion of the mucosa. Thin bloody fluid is present in considerable quantities within the lumen of the intestines. The mucosa of the large intestine is slightly congested. The pancreas is decidedly swollen by interlobular edema. There is a considerable amount of hemolysis.

TABLE 17

89 per cent blood volume exchange; fatal shock; Dog 18–5; experiment 101

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange	5.5	4.4	1.1	2.2	0.24	60	
Immediately after	2.1	1.7	0.4	1.7	0.11	41	
15 minutes after	2.4	1.8	0.6	2.2	0.11	53	
3 hours					0.13	54	
5 hours	3.6	2.6	1.0	2.2	0.21	56	Death

The refractometric estimation of serum proteins was not carried out. Nitrogen estimation by the Kjeldahl method showed that the total plasma proteins decreased as a result of the exchange from 4.9 per cent to 2.0 per cent. The fibrin content of the plasma fell from a level of 0.44 per cent to a level of 0.19 per cent as a result of the exchange. The value at the end of 15 minutes was 0.15 per cent, and 0.24 per cent at autopsy.

From a perusal of many experiments in this paper it is evident that there are wide individual variations in the susceptibility of different dogs to the plasma depletion. But each individual dog will usually react with considerable uniformity to a repeated plasmapharesis of unit volume if sufficient time is allowed between experiments for complete recovery. This is noted in tables 14 and 15 which give data from two experiments performed on the same animal at 3 weeks interval. If the second or succeeding exchanges are larger in amount

we may expect to record increasing degrees of intoxication and finally severe or fatal shock. This fact is illustrated by the preceding experiment (no. 76) in which two previous plasma depletions had no ill effects. The first one was an exchange of only 96 per cent with no signs of intoxication. The second exchange was slightly larger (118 per cent) and caused a slight intoxication. The final exchange of 178 per cent caused a prompt and fatal intoxication with the characteristic post-mortem findings described in fatal shock.

The substitution of serum for Locke's solution in plasma depletion (fibrinpharesis)

Experiment 323. (See table 18). 144 per cent exchange.

Dog 19–74. Adult female mongrel terrier. Weight 16 pounds.

August 2. Under ether anesthesia 1050 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 1100 cc. of a serum corpuscle mixture were injected into the right femoral vein. This serum corpuscle mixture consisted of 550 cc. of packed dog corpuscles washed twice with sterile

TABLE 18

144 per cent blood volume exchange; substitution of serum for Locke's solution in plasma depletion; no clinical shock; dog 19–74; experiment 323

TIME	TOTAL SERUM PROTEINS PER CENT	HEMATOCRIT RED CELL PER CENT	FIBRIN IN PER CENT
Before exchange	6.3	58	0.60
Immediately after	5.4	49	0.08
15 minutes after	5.8	54	
3 hours		50	0.30
24 hours	5.3	45	0.50

calcium-free Locke's solution in the customary way, to which was added an equal amount of serum. The serum for this purpose was obtained by drawing into large centrifuge tubes blood from normal dogs. After the process of clotting was completed the tubes were centrifugalized and the supernatant serum withdrawn. The duration of the exchange was 6 minutes. Ether anesthesia lasted 1 hour. The rectal temperature fell 2½°C. as a result of the procedure but returned to the original level within the space of about 2 hours. The arterial tension was good at the end of the exchange. There was at no time any definite impairment in the quality of the pulse. The animal showed no signs of shock.

August 3. Dog in excellent condition.

The substitution of serum for Locke's solution in the plasma depletion (fibrinpharesis)

Experiment 324. 176 per cent exchange. Female bull-terrier pup (3 months old). Weight 15 pounds.

August 5. Under ether anesthesia 1200 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 1275 cc. of serum corpuscle suspension made up as described in experiment 323 were injected into the right femoral vein. The duration of the exchange was 15 minutes. Ether

anesthesia lasted 40 minutes. The rectal temperature was depressed about 3°C. for a periof of about 2 hours. The animal remained quiet for a period of 1 hour following the exchange, the power of attention being, however, good at all times. At the end of this time the animal was in excellent condition. The hematocrit values fluctuated but slightly as a result of the experimental exchange. The fibrin content of the plasma fell from its normal level of 0.43 to 0.30 per cent 3 hours after the exchange. The reading after 24 hours was 0.65 per cent.

The two preceding experiments (table 18, expers. 323 and 324) bring out several important facts. The experimental manipulation of the red cells and the actual exchange of one mass of red cells for another are not responsible for the intoxication. In these two experiments we employed washed red cells from normal dogs prepared exactly as described for other experiments. These cells were suspended *not in Locke's solution* but in the proper amount of *fresh normal dog serum.* These large exchanges then did not wash out any serum proteins but did remove much of the fibrin. These experiments serve as good controls of the operative procedures. These large exchanges gave no evidence of any resultant intoxication. The last one especially (exper. 324) was a particularly large exchange (176 per cent) and done upon a young dog. Our experience shows that young animals as compared with adults are more sensitive to the shock of plasma depletion.

One hundred and fifty per cent exchange using washed corpuscles suspended in dialyzed serum

Experiment 327. (See table 19).

One thousand cubic centimeters of blood were drawn from normal dogs, poured immediately into large centrifuge tubes and allowed to clot. The clot formed in each tube was freed from the side of the tube and the tube centrifugalized. The supernatant serum was removed. Three hundred cubic centimeters of this serum were then placed in 15 celloidin sacs which were then immersed in 5,000 cc. of Locke's solution containing no calcium or glucose and made about 10 per cent more concentrated than normal in order that the increased osmotic pressure might in part overcome the tendency of the serum proteins to dilute themselves by attraction of water from the surrounding fluid. After dialysis had proceeded for 4 hours the modified Locke's solution was replaced by 10,000 cc. more of fresh solution of the same constitution. Dialysis was then continued for 11 hours, at the end of which time the serum contained in the celloidin sacs had increased from 300 cc. to 450 cc. To 400 cc. of the dialyzed serum 600 cc. of dog corpuscles twice washed with calcium-free Locke's solution in the ordinary way were added. The mixture was strained and heated to 38°C.

Under ether anesthesia the entire 1000 cc. of the serum corpuscle mixture were injected into the right femoral vein of a normal short-haired bull pup weighing

12.5 pounds. Simultaneously and with moderate fluctuations in the rate of flow, 850 cc. of blood were withdrawn from the right femoral artery. Thirty-five minutes were consumed in effecting the exchange. The animal showed but little alteration in body temperature as a result of the exchange. Consciousness returned shortly after the discontinuance of the anesthetic. The animal was somewhat quiet for a period of about 45 minutes. Subsequently he was bright and apparently in very good condition.

One hundred and ninety-nine per cent exchange using washed corpuscles suspended in dialyzed serum

Experiment 329. (See table 20). Nine hundred cubic centimeters of blood were drawn from normal dogs, poured immediately into large centrifuge tubes and allowed to clot. The clot formed in each tube was freed from the side of the tube and the tube centrifugalized. The supernatant serum was removed. Three hundred and fifty cubic centimeters of this serum were then placed in 18 cel-

TABLE 19

150 per cent blood volume exchange using washed corpuscles suspended in dialyzed serum; experiment 327

SAMPLE	BLOOD SERUM READINGS IN PER CENT			
	Total protein	Albumin	Globulin	Non-protein
Of serum of perfusate:				
Before dialysis	6.2	3.8	2.4	1.7
After dialysis	3.3	2.5	0.8	1.2
Of dog perfused:				
Before exchange	5.7	3.0	2.7	1.9
Immediately after	3.8	2.1	1.7	1.9
4 hours after	4.5	2.3	2.2	2.0

loidin sacs which were then immersed in 4000 cc. of Locke's solution containing no calcium or glucose, and made about 10 per cent more concentrated than normal. After dialysis had proceeded for 5 hours the modified Locke's solution was replaced by 9000 cc. of fresh modified Locke's solution. Dialysis was then continued for 10 hours, at the end of which time the serum contained within the celloidin sacs had increased from 350 cc. to 450 cc. To 400 cc. of the dialyzed serum 600 cc. of dog corpuscles twice washed in calcium-free Locke's solution in the ordinary manner, were added. The mixture was strained and warmed to 38°C.

A normal short-haired black female mongrel terrier (no. 20-62), weighing 10.2 pounds, was anesthetized with ether and the entire corpuscle suspension was injected into the right femoral vein. Simultaneously and at the same rate 925 cc. of blood were withdrawn from the right femoral artery. The exchange was effected in 10 minutes. The temperature fell to 34.7°C. immediately following the exchange but under the influence of the heat-pad returned to 37.5°C. within a space of about 1½ hour. The animal regained consciousness within about 30

minutes following the exchange and was rather quiet for another 30 minutes, but thereafter appeared to be quite normal. The pulse was at no time markedly depressed.

The two experiments, tables 19 and 20, confirm the two preceding experiments (table 18) using fresh dog's serum. Suspension of washed red blood cells in fresh dialyzed dog serum (tables 19 and 20) gives a mixture which can be used in almost unlimited amounts to exchange with whole blood by the method adopted. This exchange is associated with no clinical shock. There is a slight lowering in the concentration of blood serum protein and of course the fibrinogen is almost completely washed out of the blood. This fibrinogen, however, can be reproduced rapidly and gives no clinical reaction as its normal content is reëstablished in the blood in a few hours.

TABLE 20

199 per cent blood volume exchange using washed corpuscles suspended in dialyzed serum; experiment 329

SAMPLE	BLOOD SERUM READINGS IN PER CENT				HEMATOCRIT RED CELL PER CENT	UREA NITROGEN PER 100 cc.	NON-PROTEIN NITROGEN PER 100 cc.
	Total protein	Albumin	Globulin	Non-protein			
						mgm.	*mgm.*
Serum of perfusate:							
Before dialysis	6.7	4.0	2.7	1.5		20	40
After dialysis	5.1	3.5	1.6	1.0		2	16
Of dog perfused:							
Before exchange	5.9	4.3	1.6	1.6	45.1		
Immediately after	4.9	3.5	1.4	1.6	57.4		

It appears from these experiments that the essential factor responsible for the "shock" is the dilution of the serum proteins which is effected by the plasma depletion. The body cells cannot tolerate this diluted medium which for them is an abnormal environment. Protoplasmic injury is readily proved and if this injury is too extensive we note a familiar sequence of events which ends with fatal "shock." One may point out the narrow line which delimits a mild injury due to this plasma dilution from a severe or lethal injury and at times the reaction almost approaches the "all or none law." The change in urinary nitrogen following a moderate reaction and plasma depletion may be almost zero but following a severe or almost fatal shock due to plasma depletion we may observe a rise in urinary nitrogen on the day following which amounts to 100 to 200 per cent increase over nor-

mal. This indicates a serious injury of protein substance in the body. In a fatal plasmapharesis we may note a rapid increase in the blood non-protein nitrogen which may show over 100 per cent rise within 3 to 4 hours.

PLASMAPHARESIS COMPLICATED BY KNOWN TISSUE INJURY

In the large table 21 are collected a number of experiments to show that the presence of *injured liver cells* will predispose an animal to severe or lethal shock following a control or standard plasmapharesis. The control experiments show little or no shock following the plasma depletion of a given volume. But the same exchange performed after chloroform or phosphorus usually results in fatal shock. These experiments are in contrast to those in table 28, which presents the results of plasma depletion associated with cell injury of the kidney, pancreas and intestine. Injured cells of these organs do not modify the reaction following a standard plasmapharesis.

The three following experiments (tables 22, 23 and 24) illustrate in detail the reaction which follows plasmapharesis when preceded by chloroform anesthesia to insure a certain amount of liver necrosis and injury. The first of this group (table 22) gives a control plasmapharesis to prove that the plasma depletion alone was not responsible. The amount of liver injury was not extreme and could be tolerated by any normal animal with no clinical reaction. Note other experiments with controls in table 21.

The emergency reaction which makes possible a rapid replacement of the washed out serum proteins shows in all these experiments. The presence of the injured liver and the development of fatal shock does not modify the usual reaction by which a considerable amount of serum proteins is thrown into the circulation. This may suggest that this reaction is not purely a functional reflex but perhaps a physical phenomenon in which we see a simple exchange of protein between body cells and the circulating blood plasma—a simple washing out of a given substance related to the serum proteins which is normally present in certain body cells.

TABLE 21

Liver injury predisposes to fatal shock after plasma depletion

DOG NUMBER	POISON	BLOOD VOLUME EXCHANGE		SHOCK	BLOOD SERUM PROTEINS IN PER CENT				REMARKS
		Per cent	Time in minutes		Before exchange	End of exchange	15 minutes after	24 hours after	
17-212	0	141	12	None	5.5	2.6	2.5	3.9	
17-212	Chloroform (1 hour)	144	13	Fatal	5.5	2.1	3.4		
18-6	0	118	17	Slight	7.1	4.2		4.8	
18-6	Chloroform (1½ hour)	175	12	Fatal	6.6	1.7	2.5		
18-9	0	170	12	Moderate	5.6	2.0	2.9	4.2	
18-9	Chloroform (1½ hour)	198	10	Severe	4.8	2.0	2.4	4.0	Drug given 48 hours previously
17-215	0	159	10	Slight	6.2	1.3	2.2	4.1	
17-215	Phosphorus (17.5 mgm.)	140	7½	Fatal	4.9	1.2	1.2		Drug given 40 hours previously
17-233	0	67	5½	Slight					
17-233	Phosphorus (14 mgm.)	118	15	Fatal	4.9	0.7	2.1		
18-34	0	75	6	Moderate	5.3	2.9	3.4	4.3	
18-34	Phosphorus (5.2 mgm.)	77	6½	Fatal	5.6	3.1	3.5		
18-7	0	96	12	None	5.5	2.7	3.6		
18-7	Phosphorus (11 mgm.)	118	7	Moderate	6.5	1.4	3.2	3.7	Drug given 5 hours later
18-66	0	91	9	Moderate	6.2	3.2	3.8	4.3	
18-66	Hydrazine (140 mgm.)	82	9	Fatal	5.7	4.2			
18-68	0	90	9	Moderate	5.5	2.8	3.5	4.8	
18-68	Hydrazine (100 mgm.)	88	9	Moderate	4.9	2.6	3.3	4.6	
18-68	Hydrazine (100 mgm.)	95	7	None	5.2	2.3	3.1		

Poison given in every experiment 18 to 26 hours before plasmapharesis unless otherwise noted.

Plasmapharesis before and after chloroform

Experiment 71. (See table 22). 198 per cent exchange.

Dog 18–9. Female bull-terrier pup. Weight 12 pounds.

August 2. Plasmapharesis, 170 per cent exchange in 12 minutes. Little if any intoxication.

August 4. Chloroform anesthesia for 1½ hour, undergoing recovery without clinical signs of injury.

August 6. Animal appears to be in excellent condition. Under ether anesthesia 1081 cc. of blood were withdrawn from the left femoral artery. At the same time and at the same rate 1081 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The exchange was effected in a space of 10 minutes. There was a steady fall in blood pressure and in the volume of the pulse. An extreme grade of depression was present within a half-hour and the heart beat was barely palpable 3 hours after the exchange. The rectal temperature had fallen at this time to a level of 30°C. From this point on slow but gradual improvement was noted. Eventually complete recovery occurred.

TABLE 22

198 per cent blood volume exchange; plasmapharesis following chloroform; dog 18–9; experiment 71

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange	4.8	3.2	1.6	1.7	
Immediately after	2.0	1.2	0.8	1.3	
15 minutes after	2.4	1.5	0.9	1.6	Profound shock
2nd day	4.0	2.4	1.6	2.3	
3rd day	4.9	3.1	1.8	2.0	Good recovery

Plasmapharesis following chloroform

Experiment 72. (See table 23). 175 per cent exchange.

Dog 18–6. Young-adult female Dachshund. Weight 13.4 pounds. Blood volume on July 1 (by dye method) was 761 cc.

On July 24 a 118 per cent exchange was performed in 8 minutes with little or no shock.

August 7. Chloroform anesthesia for 1½ hour.

August 8. Under ether anesthesia 1065 cc. of blood were withdrawn from the left femoral artery. Simultaneously an equal quantity of Locke's corpuscle suspension was injected into the left femoral vein. The duration of the exchange was 12 minutes. The rectal temperature showed little immediate alteration as a result of the exchange. However a gradual fall in temperature soon appeared, the level of 36.4°C. being reached at the time of death. 1½ hour later. The arterial pulse became slow and weak almost at once following the exchange. The respiration was gasping in character within 15 minutes following the exchange

and a profound degree of depression existed. The condition gradually became worse and death occurred 1½ hour following the exchange.

Autopsy: The blood drawn from the heart shows no tendency to clot within the space of 24 hours. Even such blood when placed in contact with fresh tissues shows no tendency to clot. The thoracic organs are negative. The spleen is somewhat enlarged and the Malpighian bodies are approximately twice their normal size. The pancreas is slightly congested. The liver is congested. A considerable amount of necrosis due to chloroform injury is seen in the centers of the lobules. Histological examination shows a fairly extensive central hyaline necrosis involving about one-half of each liver lobule. There is some fatty de-

TABLE 23

175 per cent blood volume exchange; plasmapharesis following chloroform; dog 18–6; experiment 72

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange	6.6	3.6	3.0	1.7	
Immediately after	1.7	0.6	1.1	1.6	
15 minutes after	2.5	1.5	1.0	1.6	
1½ hours after					Death

TABLE 24

144 per cent blood volume exchange; plasmapharesis following chloroform; dog 17–212; experiment 78

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMOGLOBIN PER CENT (SAHLI)	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange	5.5	3.1	2.4	1.6	0.33	97	
Immediately after	2.1	1.4	0.7	1.5	0.13	92	
15 minutes after	3.4	2.4	1.0	1.3	0.18	92	
2 hours	5.5	3.6	1.9	1.7	0.12	93	Death

generation of the liver cells in the mid-zone of each lobule. This lesion could be tolerated by a dog with few if any clinical symptoms. The kidneys show considerable engorgement of the medulla. The mucosa of the entire gastro-intestinal tract is pink and a considerable amount of mucus is contained in the lumen.

Plasmapharesis following chloroform

Experiment 78. (See table 24). 144 per cent exchange.

Dog 17–212. Young adult female spaniel. Weight 14.5 pounds. Blood volume on July 19 (by dye method) was 632 cc.

An exchange of 94 per cent was performed on July 23, and another of 141 per cent on August 1, with practically no signs of shock in either case.

August 21. Chloroform anesthesia for 1 hour.

August 22. Animal appears to be in excellent condition. Under ether anesthesia 950 cc. of blood were withdrawn from the left carotid artery. Simultaneously 950 cc. of Locke's corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 13 minutes. The rectal temperature gradually fell about 4°C. from the normal level in the 2 hours following the exchange. Fluid blood-stained feces were noted at the end of the first hour following the exchange. The dog went into profound shock and died 2 hours following the exchange.

Autopsy: The thoracic organs are negative. The spleen and kidneys show considerable congestion. The liver is large and congested. In gross there is evidence of chloroform injury and histological sections show an early stage of chloroform necrosis which involves liver cells in the centers of lobules. This injury is slight in degree and by itself would give no clinical reaction in the dog. The mucosa of the stomach and small intestines is thickened and dark red in color. A considerable excess of mucus and fluid material is present in the intestinal lumen.

The following experiment (table 25, exper. 95) is complete in that a control plasmapharesis causes only a little intoxication. The dose of phosphorus is less than one-half a lethal dose and would be tolerated by a normal dog without clinical symptoms. The combined phosphorus injury and a second plasmapharesis causes a typical lethal shock.

It may be noted that the *hematocrit* figures which are complete for this experiment show no evidences of any definite change in red cell plasma ratio. The same observation holds in the chloroform experiments. When we review all these shock experiments and compare them with duplicate experiments in which no shock appears we cannot assign any of these reactions to a process of concentration of the blood. In certain experiments there is a rise in cell hematocrit taken 15 minutes and 1 to 4 hours after the exchange. But the same rise is noted at the very end of the exchange and the correct explanation we believe is to be found in the red cell mixture introduced. This red cell mixture contains more red cells per cubic centimeter than the blood of the dog under observation. There is a constant fall of hematocrit on the 2nd day but we believe this is to be explained by the disintegration of the red cells which have been injured in the routine process of washing in Locke's solution.

The second phosphorus experiment (no. 73) is given in table 21. The control plasmapharesis caused no reaction but the same exchange

preceded by a small dose of phosphorus was fatal in 2 hours. In another experiment the plasmapharesis was *followed* by a large dose of phosphorus. The intent was to follow the curve of protein regeneration as influenced by this drug which causes such characteristic liver injury.

Plasmapharesis following phosphorus

Experiment 95. (See table 25). 77 per cent exchange.

Dog 18–34. Female bull pup. Weight 17.1 pounds. Blood volume (by dye method) was 805 cc.

September 5. The usual plasmapharesis with 80 per cent exchange was completed in 4 minutes without the production of shock.

September 26. Plasmapharesis with 75 per cent exchange was carried out in 6 minutes. There was a certain amount of clinical depression, but no serious shock.

TABLE 25

77 per cent blood volume exchange; plasmapharesis following phosphorus; dog 18–34; experiment 95

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange	5.6	4.2	1.4	2.0	0.16	55	
Immediately after	3.1	2.3	0.8	1.3	0.26	54	
15 minutes after	3.5	2.6	0.9	1.6	0.33	58	
3 hours	3.6	2.6	1.0	1.7	0.30	54	
6 hours	4.0	2.9	1.1	1.5	0.29	54	Profound shock

October 16. Phosphorus, 5.2 mgm. in olive oil, given subcutaneously.

October 17. Animal appears to be in excellent condition. Under ether anesthesia 600 cc. of blood were withdrawn from the right carotid artery. Simultaneously 600 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 6.5 minutes. The arterial tension was fair at the end of the exchange, but was very poor at the end of another half-hour. Although showing a considerable amount of prostration, the animal was conscious for several hours. The animal was in profound shock at the end of 6 hours, and was found dead 12 hours after the exchange. The body was still somewhat warm, but rigor mortis was fairly well developed.

Autopsy: The tissues at the root of the lungs and about the smaller bronchi within the lung are somewhat edematous. The spleen is practically normal. The liver is quite pale and slightly translucent. Its lobulation is indistinct. Kidneys show slight congestion along the cortico-medullary line. The stomach is negative. The mucosa of the small intestine is thickened and moderately congested. A considerable amount of mucus is found in the lumen.

Histological sections: The liver shows very little evidence of cell injury. There are a few pale nuclei, but the fatty change so common in the cell protoplasm after large doses of phosphorus is absent. A slight increase in the leucocytes in the liver capillaries is noted. Spleen, pancreas, lung and intestines are negative.

Plasmapharesis following phosphorus

Experiment 73. 140 per cent exchange.

Dog 17–215. Young adult female fox-terrier. Weight 17.4 pounds. Blood volume on July 19 (by dye method) was 858 cc.

July 31. An exchange, 159 per cent, was performed in 10 minutes, causing no definite signs of shock.

August 7. Phosphorus, 17.5 mgm. in olive oil, was given subcutaneously.

August 9. Under ether anesthesia 1105 cc. of blood were withdrawn from the left femoral artery. Simultaneously 1105 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 8 minutes. Profound depression was in evidence almost immediately. There was a very marked weakening in the pulse. Bloody feces appeared within an hour following the exchange. Death followed the exchange by 2 hours. There was a gradual fall in rectal temperature of 4°C. during the course of the experiment. The total blood serum proteins fell from 4.9 per cent to 1.2 per cent as a result of this exchange. Other figures are not available because of loss of material.

Autopsy: Blood drawn from the heart immediately after death does not clot even on the addition of tissue juices. The thoracic organs are negative. The spleen is dark red and enlarged to about twice the normal size. The Malpighian bodies are large, distinct and opalescent. The liver is somewhat enlarged. The centers of the hepatic lobules are dull red while the peripheral portions are yellowish. The stomach shows distention of the superficial veins and moderate engorgement of its mucosa. The mucosa of the duodenum and upper portion of the jejunum is markedly engorged. The mucosa of the lower portion of the small intestine is but slightly reddened, while the large intestine is negative. The pyramids of the kidneys are slightly engorged. A few scars are seen in the cortex.

Histological sections: Liver shows early changes in cell protoplasm, especially small fat droplets. This dose of phosphorus should give a severe but not lethal liver injury. The injury at this stage is very inconspicuous. There is a notable interstitial edema of the pancreas. Other organs are negative.

Plasmapharesis followed by phosphorus

Experiment 65. (See table 26). 118 per cent exchange.

Dog 18–7. Young adult female mongrel terrier. Weight 10.9 pounds. Blood volume (by dye method) was 530 cc.

July 19. An exchange, 96 per cent, performed in 12 minutes caused no shock.

July 26. Under ether anesthesia 583 cc. of blood were withdrawn from the femoral artery. Simultaneously 583 cc. of Locke's corpuscle suspension were

injected into the femoral vein. The duration of the exchange was 7 minutes. With the exception of a fall of about 1°C. in rectal temperature there was little obvious disturbance as a result of the exchange. About 5 hours after the exchange 11 mgm. of phosphorus dissolved in olive oil were injected subcutaneously. On the following day the animal appeared rather quiet, but not otherwise disturbed. The food was not eaten for several days and on August 1 the dog weighed 9.25 pounds. Complete recovery occurred several days later.

TABLE 26

118 per cent blood volume exchange; plasmapharesis followed by phosphorus; dog 18–7; experiment 65

TIME	BLOOD SERUM READINGS IN PER CENT			
	Total protein	Albumin	Globulin	Non-protein
Before exchange	6.5	5.7	0.8	2.0
Immediately after	1.4	0.3	1.1	1.5
15 minutes after	3.2	1.8	1.4	1.7
2nd day	3.7	2.6	1.1	2.5
3rd day	3.8	2.4	1.4	2.3
5th day	5.1	2.0	3.1	1.5
10th day	4.3	2.0	2.3	1.8

TABLE 27

88 per cent blood volume exchange; plasmapharesis following hydrazine sulfate; dog 18-68; experiment 108

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	4.9	3.2	1.7	1.7	0.31	27
Immediately after	2.6	1.7	0.9	1.6	0.26	42
15 minutes after	3.3	2.3	1.0	1.4	0.31	56
3½ hours	3.7	2.6	1.1	1.9	0.40	41
2nd day	4.6	3.2	1.4	1.7		
3rd day	4.7	3.3	1.4	1.6		33
6th day	5.2	2.8	2.4	2.3		

Plasmapharesis following hydrazine sulfate

Experiment 108. (See table 27). 88 per cent exchange.

Dog 18–68. Female mongrel bull pup. Weight 14.3 pounds.

November 21. An exchange of 90 per cent performed in 9 minutes caused a moderate grade of shock.

November 27. Hydrazine sulfate, 100 mgm., injected subcutaneously.

November 28. Under ether anesthesia 575 cc. of blood were withdrawn from the left femoral artery. Simultaneously 575 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 9

minutes. There were at no time any definite signs of depression. The arterial tension remained moderately good throughout. There was a fall in rectal temperature of about 1°C. during the exchange. There was, however, a prompt return—in fact to a point slightly above the original temperature for a period of several hours, after which the temperature returned to the normal level.

The preceding experiment (table 27) gives some evidence that hydrazine sulfate as a liver poison differs somewhat when compared with chloroform or phosphorus. This dog (18–68) showed no less reaction to the control plasmapharesis than to the same exchange preceded by hydrazine sulfate. In another experiment, however, (table 21, exper. 109) we see the familiar reaction with fatal shock due to a combined plasmapharesis and hydrazine poisoning. The control of the plasmapharesis showed a definite but not severe intoxication.

The preceding table (table 28) lists the reactions which follow a plasmapharesis combined with cell injuries of various other organs and tissues. The control plasma depletion on the same dog is given in each experiment. When the remarkable reaction and fatal shock were noted in the phosphorus and chloroform experiments we suspected at once that any cell injury might render the experimental animal more sensitive to the shock of plasmapharesis. The experiments in table 28, however, show that such is not the case.

The *kidney* epithelium was injured by administration subcutaneously of uranium nitrate in suitable dosage. Two experiments show identical reactions in the control plasma depletion as in the plasmapharesis following the administration of uranium nitrate. One experiment (dog 18–35) shows a fatal reaction but there are many unusual features which we cannot explain—see table 30 below for details.

Pancreas injury is represented by only a single experiment but this is very clean-cut. The pancreas was injured by the injection of bile into its main duct. The control exchange gives the same negative reaction as the plasma depletion preceded by the acute pancreatitis.

The *Roentgen-ray* is able to cause a specific and extensive injury to the lymphatic tissue but especially to the *epithelium* of the *small intestine* as has been shown by the work of Hall and Whipple (6). This injury and consequent intoxication develops to its maximum on the 4th day following an exposure over the abdomen. A plasmapharesis done 24 hours after X-ray exposure gives the same reaction as in the control period. This shows that even the extensive injury which in a fatal case of X-ray intoxication involves the greater part of the epithelium of the small intestine does not modify the shock of plasmapharesis. This is in striking contrast to the liver injury.

TABLE 28

Kidney, pancreas and intestinal epithelium injury does not predispose to shock after plasma depletion

DOG NUMBER	POISON	BLOOD VOLUME EXCHANGE		SHOCK	BLOOD SERUM PROTEINS IN PER CENT			
		Per cent	Time in minutes		Before exchange	End of exchange	15 minutes after	24 hours after
18-35	0	74	14	None	5.5	3.7	3.8	
18-35	Uranium (5 mgm.)	72	14	Fatal	6.3	3.9	4.6	5.8
18-48	0	104	2	Slight	6.4	2.1	3.2	4.5
18-48	Uranium (6 mgm.)	77	4	None	6.1	3.1	4.1	4.6
18-66	0	91	9	Moderate	6.2	3.2	3.8	4.3
18-66	Uranium (8 mgm.)	96	11½	Moderate	5.1	2.9	3.2	4.8
18-65	0	100	13	Slight	5.6	3.0	2.9	4.6
18-65	Pancreatitis	94	15	None	5.3	3.3	3.9	4.8
18-68	0	90	9	Moderate	5.5	2.8	3.5	4.8
18-68	X-ray* (175 M.A.M.)	90	7	Moderate	5.9	3.2	3.9	4.7
18-65	0	100	13	Slight	5.6	3.0	2.9	4.6
18-65	X-ray (200 M.A.M.)	105	9	None	5.7	2.9	3.3	4.7

Injury given in every experiment 20 to 24 hours before plasmapharesis with exception noted: * X-ray given 45 hours before plasmapharesis.

TABLE 29

77 per cent blood volume exchange; plasmapharesis following uranium nitrate; dog 18-48; experiment 99

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	6.1	3.4	2.7	1.7		
Immediately after	3.1	1.9	1.2	1.4		
15 minutes after	4.1	2.6	1.5	1.5	0.25	61
2 hours	4.0	2.6	1.4	1.5	0.25	36
6 hours	4.7	2.9	1.8	1.8	0.32	29
2nd day	4.6	3.0	1.6	2.0	0.45	41
6th day	5.2	3.4	1.8	2.2	0.42	41
8th day	5.1	3.4	1.7	2.2	0.29	46
9th day	5.1	3.4	1.7	2.1		
11th day	5.2	3.5	1.7	2.4	0.33	43

Plasmapharesis following uranium nitrate

Experiment 99. (See table 29). 77 per cent exchange.

Dog 18–48. Female bull pup. Weight 14.3 pounds.

October 4. Exchange of 99 per cent performed in 12 minutes with the production of but slight grade of shock.

October 18. An exchange of 104 per cent in 2 minutes was performed with very little shock.

October 31. Uranium nitrate, 6 mgm., given subcutaneously.

November 1. Animal seems to be in excellent condition. Under ether anesthesia 500 cc. of blood were withdrawn from the right carotid artery. Simultaneously 500 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 14 minutes. At no time during the experiment was there any definite sign of shock. The arterial tension was good at the end of the exchange but shortly thereafter fell slightly for a period of several hours. The rectal temperature fell nearly 2°C. during the exchange but returned to normal in the course of several hours.

Plasmapharesis before and after uranium nitrate

Experiment 97. (See table 30). 72 per cent exchange.

Dog 18–35. Female bull pup. Weight 16.7 pounds.

September 12. An exchange of 80 per cent was performed in 7 minutes, causing slight intoxication.

October 3. An exchange of 73 per cent was effected in 14 minutes, with no signs of intoxication.

October 22. An aqueous solution of 10.4 mgm. *uranium nitrate* was injected subcutaneously.

October 23. Animal appears to be in good general condition. Under ether anesthesia 545 cc. of blood were withdrawn from the left carotid artery. Simultaneously and with no great variation in rate 545 cc. of Locke's corpuscle suspension were injected into the left jugular vein. The duration of the exchange was 13 minutes. The rectal temperature fell from 39.5 to 37°C. within a space of 4 hours. Drowsiness soon appeared, the pulse diminished in volume after about 3 hours and was decidedly poor several hours later. Bloody feces were first noted about 11 hours following the exchange. The next morning the condition was worse and a considerable amount of bloody feces had been passed. The animal was suffering from convulsive attacks. The temperature was 37°C. Death occurred about 20 hours following the exchange.

Autopsy: The tissues are definitely jaundiced. The thoracic organs are essentially negative. The spleen is large, the edges rounded, and on section presents definite congestion. The liver shows only indistinct lobulation. The kidneys are definitely engorged. The stomach is negative except for a slight amount of engorgement of the mucosa. The intestinal mucosa is decidedly engorged with blood, the condition being more marked in the lower portion of the gut.

Histological sections: Kidneys show much epithelial degeneration and necrosis involving particularly the convoluted tubules. There are numerous hyaline, hemoglobin and blood casts in the collecting tubules. Other organs present nothing of interest.

The preceding experiments (tables 29 and 30) are in conflict. The first one (exper. 99) shows a negative reaction when a standard plasma depletion is combined with kidney injury due to uranium nitrate. The other experiment (exper. 97) shows a fatal reaction but it is atypical. The shock did not develop quite as usual and the dog seemed about to recover. When the shock of plasmapharesis is tolerated for 12 hours the dog usually recovers and appears normal and active within 24 hours. This dog on the day after the experiment developed convulsions and died. There was jaundice and at autopsy signs of blood destruction. The histological sections give evidence of considerable epithelial injury in the secreting tubules of the kidney. That this kidney injury played a part in the late death is highly probable but

TABLE 30

72 per cent blood volume exchange; plasmapharesis following uranium nitrate; dog 18–35; experiment 97

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Nonprotein			
Before exchange	6.3	4.7	1.6	1.7	0.32	22	
Immediately after	3.9	3.0	0.9	1.5	0.14	25	
15 minutes after	4.6	3.5	1.1	1.4	0.16	42	
2½ hours	5.1	3.7	1.4	1.4	0.19	38	
5 hours	5.2	3.9	1.3	1.4		37	
2nd day	5.8	4.0	1.8	1.8	0.32	30	Death

it is clear that the shock of plasmapharesis was atypical. In view of the other experiments we do not attach too much importance to a single atypical experiment which appears to be at variance with the general type reaction.

Acute pancreatitis followed by plasmapharesis

Experiment 102. (See table 31). 94 per cent exchange.

Dog 18–65. Female bull pup. Weight 17 pounds.

November 7. An exchange of 100 per cent effected in 13 minutes caused a very moderate grade of shock.

November 13. Under ether anesthesia laparotomy was performed and 10 cc. of sterile bile injected by means of a hypodermic needle into the pancreatic duct. The wound was closed. It is known that this will cause an intense diffuse hemorrhagic pancreatitis.

November 14. The animal is lively and apparently in quite good condition. Under ether anesthesia 730 cc. of blood were withdrawn from the left femoral artery. Simultaneously 730 cc. of Locke's corpuscle suspension were injected into the left femoral vein. Seven minutes were consumed in effecting the exchange. At no time was there any definite evidence of intoxication. Refer to experiment 106, table 33, for autopsy.

The preceding experiment (table 31) is complete and supports the two following experiments with X-ray injury. This pancreas was severely injured by an injection of bile into the pancreatic duct. We have frequently produced an acute hemorrhagic pancreatitis in this way and the injury may be sufficient to produce lethal intoxication by itself. That extensive injury was done this pancreas is afforded by

TABLE 31

94 per cent blood volume exchange; acute pancreatitis followed by plasmapharesis; dog 18–65; experiment 102

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange	5.3	3.6	1.7	2.2	0.49	36
Immediately after	3.3	2.5	0.8	1.6	0.29	54
15 minutes after	3.9	2.9	1.0	1.4	0.22	58
3 hours	4.6	3.1	1.5	1.4	0.40	53
8 hours	4.3	2.9	1.4	2.2	0.34	41
2nd day	4.8	2.9	1.9	2.2	0.29	40
3rd day	4.6	2.6	2.0	2.2	0.25	35
4th day	4.0	2.8	1.2	2.8	0.35	32
6th day	5.0	2.8	2.2	2.9	0.31	27
7th day	5.0	3.7	1.3	2.7	0.40	29

examination of the autopsy record of this dog (exper. 106, table 33 below) which shows a scarred, indurated pancreas speckled with old fat necroses. Yet these injured pancreas cells did not modify the reaction following a controlled plasma depletion.

Plasmapharesis following sublethal X-ray exposure

Experiment 111. (See table 32). 90 per cent exchange.

Dog 18–68. Female bull pup. Weight 14 pounds.

November 21. An exchange of 90 per cent effected in 9 minutes caused a very moderate grade of shock.

December 10. X-ray exposure over abdomen in 4 quadrants, 2 mm. aluminum filter, 175 milliampere minutes, with 9 inch spark gap. Distance from target to skin is 10 inches.

December 12. Animal seems to be in excellent condition. Under ether anesthesia 575 cc. of blood were withdrawn from the left carotid artery. Simultaneously 575 cc. of Locke's corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 7 minutes. There was but a very slight amount of depression. The arterial tension was good at the end of the exchange, but became of poorer quality in the course of the next 2 hours. The animal walked about occasionally. The rectal temperature fell about 1°C. during the course of the experiment.

Lethal dose of X-ray followed by plasmapharesis

Experiment 106. (See table 33). 105 per cent exchange.

Dog 18–65. Female bull pup. Weight 16.8 pounds.

November 7. An exchange of 100 per cent effected in 13 minutes caused a very moderate grade of shock.

November 14. An exchange of 94 per cent following acute experimental pancreatitis produced no definite signs of intoxication (see exper. 102, table 31).

November 21. X-ray exposure over abdomen in 4 quadrants, 2 mm. aluminum filter, 200 milliampere minutes, with 9 inch spark gap. Distance from target to skin is 10 inches.

November 22. The animal appears to be in good condition. Under ether anesthesia 800 cc. of blood were withdrawn from the right carotid artery. Simultaneously 800 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 9 minutes. The animal showed a very slight amount of depression as a result of the exchange. The pulse remained fair throughout. There was but slight depression of the rectal temperature (1°C.).

November 23. The animal seems a bit weak. Has vomited material containing some intestinal worms.

November 24. The dog appears somewhat better.

November 25. Quite weak. Mucous, blood-tinged feces. Some vomiting. Refuses food.

November 26. Death occurred in the afternoon. Autopsy performed at once.

Autopsy: Thoracic organs negative. Blood drawn from the heart clots normally. Blood urea nitrogen is 32.5 mgm. per 100 cc. Spleen is small, light red, with an increase in fibrous tissue. Liver is pale and anemic. Pancreas: lower arm is hard, shrunken and scarred; the result of the pancreatitis described in experiment 102, table 31. Many old fat necroses are present. The upper arm is scarred but appears more nearly normal. Kidneys and adrenals are negative. Gastro-intestinal tract shows only a few scattered patches of congestion.

Histological sections: The pancreas shows extensive fibrosis—the result of the preceding acute injury. The small intestine shows much epithelial injury in its deep crypts. There is some evidence of epithelial regeneration as well as degeneration. This epithelial injury we believe to be the immediate cause of death. Other organs present nothing of interest for this experiment.

The two preceding experiments (tables 32 and 33) show the influence of X-ray injury of the body cells upon a standard plasmapharesis.

The first experiment (table 32) shows the result of a sublethal exposure to the X-rays. The reaction to the plasma depletion in a control exchange is not modified.

The second experiment (table 33) shows a reaction recently described in some detail by Hall and Whipple (6). This reaction is due

TABLE 32

90 per cent blood volume exchange; plasmapharesis following sublethal x-ray exposure; dog 18-68; experiment 111

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange	5.9	4.2	1.7	2.2	
Immediately after	3.2	2.6	0.6	1.7	
15 minutes after	3.9	3.0	0.9	1.8	No shock
3 hours	4.4	3.1	1.3	1.9	
5½ hours	4.5	3.3	1.2	1.9	
2nd day	4.7	3.3	1.4	2.7	
4th day	4.5	3.0	1.5	2.6	Dog normal

TABLE 33

105 per cent blood volume exchange; lethal dose of x-ray followed by plasmapharesis; dog 18-65; experiment 106

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange	5.7	3.7	2.0	2.1	0.45	33	
Immediately after	2.9	2.3	0.6	1.7	0.22	44	
15 minutes after	3.3	2.6	0.7	1.7	0.27	34	Slight shock
4 hours	4.1	2.8	1.3	1.8	0.28	37	
7 hours	4.2	3.4	0.8	1.5			
2nd day	4.7	3.5	1.2	2.1	0.47	26	
3rd day	3.9	2.1	1.8	3.0		25	X-ray intoxication
4th day	4.0	1.7	2.3	3.8	0.72	30	
5th day	5.9	3.0	2.9	3.1	0.95		Death

to a lethal dose of the X-ray—in this instance 200 milliampere minutes, 90 kilo volts, given over the abdomen. Death on the 4th day with the usual blood-tinged feces and prostration is the usual reaction in these animals given a lethal exposure of the X-ray. Details of this reaction and the post-mortem findings may be found in the publica-

tion just noted. The control plasmapharesis which was done 24 hours after the X-ray exposure did not give any symptoms of intoxication and this reaction due to the plasma depletion was not modified by the presence of a great amount of injured epithelium of the small intestine. We have many experiments to show that on the second day after X-ray exposure *epithelial injury and necrosis* can be made out histologically in the small intestine. These cells will undergo rapid autolysis under a variety of conditions and it is quite remarkable that the plasmapharesis should not be modified by this great mass of injured epithelial cells.

DISCUSSION

In some earlier experiments, Kerr, Hurwitz and Whipple (3), it was noted that the presence of liver injury or liver cell necrosis made a given animal much more vulnerable to the injury and consequent shock which followed a given plasma depletion. Using single rapid depletion by the method described in the experiments cited above similar results were observed. A number of such experiments are given in table 21 above and it will be noted that the control experiment in every case shows little or no shock following plasmapharesis, but an identical procedure if associated with slight liver injury was almost always fatal. There is apparently little or no difference in this respect between the liver injury due to chloroform and that due to phosphorus. The liver injury due to hydrazine sulfate was not studied in a sufficient number of experiments.

The interesting fact stands out that a trifling injury due to phosphorus or chloroform can be tolerated by a dog with no clinical reaction. But if at this time (24 to 48 hours after administration of the chloroform or phosphorus) we perform a plasmapharesis of small volume which was previously tolerated by the same dog with little or no intoxication, we immediately precipitate severe or fatal shock. The combination of slight liver injury and a moderate exchange (plasmapharesis) will result fatally in almost all cases. How may we explain this observation? There are many possibilities but we favor the following explanation. The chloroform or phosphorus causes an injury to many liver cells and these cells are more susceptible to other injurious agents than are the normal liver cells. A sudden change in the protein content of the blood which bathes these injured cells will react more unfavorably upon them than upon the healthy and more resistant normal liver cells. These damaged (phosphorus) and then shocked (plas-

mapharesis) liver cells form substances which are taken up by the blood and carried to all the living cells of the body. If these poisonous substances are sufficient in amount we observe the development of lethal shock. We may not assume simple intensive injury and paralysis of the *liver cells alone* because it is known that the body can tolerate complete ablation of the liver cells for a period of 5 to 7 hours (7). When we produce an intensive form of shock (plasmapharesis) we may observe death within 1.5 hour. This cannot be explained by any *local reaction* limited strictly to the liver cells.

We observe in other experiments (table 28) that cell injury of other organs (kidney, pancreas and intestine) does not modify the familiar reaction following a moderate exchange. The control and poisoning experiments give similar reactions. This indicates a peculiar relation of the liver cells to the *shock reaction* associated with plasma depletion.

SUMMARY

Bleeding a dog from a large artery and a simultaneous replacement of a red blood cell Locke's solution mixture may be called "plasma depletion" or "plasmapharesis." This procedure will rapidly wash out large amounts of plasma proteins and cause a precipitous fall in the blood plasma protein concentration.

The reaction following such procedures may be minimal or it may be lethal. In general the larger the exchange the greater the probability of lethal shock. Repeated plasma depletions carried out at intervals of days or weeks on the same animal will give uniform reactions if the volume exchange and other experimental factors are constant.

"Plasmapharesis" may be performed with washed red cells suspended in normal dog serum or fresh dialyzed dog serum. When we replace the Locke's solution in the red cell mixture by dog serum we remove completely the toxic effect of the plasma depletion. This gives control for the experimental procedure but, more important, gives strong indication that the *blood serum proteins are stabilizing or protective factors.* They are essential environmental factors of the circulating blood in its relation to the body cells. This may be the most important function of these plasma colloids.

The presence of injured cells of the kidney, pancreas or intestine does not seriously modify the expected reaction following a uniform plasmapharesis.

The presence of *injured liver cells* (chloroform, phosphorus) *does profoundly modify the expected reaction* following a unit plasmapharesis. A fatal shock reaction is almost constant following even a moderate plasma depletion preceded by liver injury.

This would indicate that the liver cells are particularly concerned in the peculiar shock reaction which may follow plasmapharesis and lowering of the blood plasma protein values. It may be that this type of "shock" is not unlike the common "surgical shock."

The evidence in our experiments gives strong support to the theory that in "shock" there is a *primary cell injury* which *precedes* the familiar *clinical reaction.*

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III. FACTORS CONCERNED IN THE PERFUSION OF LIVING ORGANS AND TISSUES

Artificial Solutions Substituted for Blood Serum and the Resulting Injury to Parenchyma Cells

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These experiments were undertaken for the purpose of investigating the formation of the serum proteins in the body. The source of the serum proteins is still a mystery in spite of indirect evidence brought out by Kerr, Hurwitz and Whipple (1) to show that the *liver* is concerned in the regeneration of new serum proteins as well as in the maintenance of the normal serum protein concentration in the circulating blood. It seems too that other organs or tissues as well as the liver must be able under emergency conditions to produce certain amounts of new serum protein. It would appear that the normal wear and tear of serum proteins must be slight as these substances can be formed only with so much difficulty when the normal level has been greatly lowered.

Theoretically, perfusion of organs should offer an ideal method of solving these problems. Using a mixture of red cells and whole serum or diluted serum or modified Locke's solution, the investigator should be able to perfuse satisfactorily the various organs or combinations of organs and tissues. A simple determination of the protein values before and after such a procedure would then give the desired information and enable one to say whether a certain organ did or did not contribute any serum proteins. Information as to whether serum proteins are used by or destroyed in these organs might also be made available.

We have been able to convince ourselves that the present methods are not satisfactory to permit of the solution of this serum protein problem just outlined above. One of the conclusions which has been forced upon us is that much of the older experimentation in the field of organ perfusion is of little or no value as regards deductions made from

such experiments which postulated a living organ or organ cells. When parenchymatous organs are perfused with Locke's solution or some modification of this solution with or without red cells, we wish to suggest the probability that the research worker is dealing not with normal cells but with cells which have been injured or destroyed by contact with the perfusate. The investigator is then perfusing a dying or dead autolysing tissue or organ. Deductions drawn from such experiments must be cautious and proper allowance in every case made for this profound injury of the parenchyma cells.

The "stabilizing value" of the blood serum proteins and the injury done the various body cells by contact with diluted plasma have been emphasized in the preceding communication. We may also note the observation of Guthrie (2) to the effect that organ transplantation is a failure if the transplanted organ is washed out with normal saline before the blood flow through the organ is reëstablished. The transplanted organ does not resume its function and we may assume that its cells were definitely injured by the short period of contact with the salt solution.

The gradual slowing of the perfusion flow through any given organ is a familiar observation and we believe it may be due in part to actual injury of cells, including the endothelium. The simultaneous development of edema is a part of the same general reaction.

From many of our earlier experiments we gained the impression that perfusion or plasmapharesis with the use of Locke's solution inflicted a destructive injury upon certain body cells and that this injury of protein was responsible for the fatal intoxication and death. Our conception was somewhat as follows: the initial injury might damage irreparably liver cells (or other body cells) and from these injured organ cells were derived poisonous substances (protein split products) sufficient to cause death. It has been pointed out that complete paralysis of all liver cells could not explain this phenomenon as ablation of the liver can be tolerated for 5 to 7 hours, while the shock following a large plasmapharesis may cause death within 2 hours.

Postulating the presence in the body of some poisonous substance referable to the perfusion or plasmapharesis, we have attempted in many of our perfusion experiments to demonstrate the presence of a poison in the perfusate at the end of any given experiment. In only one experiment (exper. 5) have we evidence for the presence of any poison under these conditions of perfusion. But we have some evidence (expers. 17 and 18) to show that a known poison of protein origin

added to a given non-toxic perfusate may be in part removed within 20 to 30 minutes' continuance of perfusion. This is evidence that a poison of colloid nature may be removed from the circulation in such experiments—so the absence of a poison in our perfusates does not negative the possibility of poison being formed by the injured cells and contributed to the blood stream or perfusate. We have further been able to show that an enormous dose of proteose-like, toxic material may be wholly removed from the blood stream within a period of 5 minutes after intravenous injection in a normal dog.

We have discussed the results of the experiments given below in the light of the plasmapharesis experiments given in the two preceding papers. It may be noted that these perfusion experiments were done *before* the plasma depletion experiments. The evidence which may be taken from our perfusion experiments is not as definite as that obtained in the later plasmapharesis experiments but these data are all in harmony. A most important fact is that *physiological perfusion* of organs is very difficult and *slight modification of the blood plasma* may have profound effect upon body cells.

DEVELOPMENT OF PERFUSION METHODS

The notion of artificial perfusion was long ago expressed by Le Gallois (3). He maintained that by artificial perfusion life might be kept up in any portion of the animal even though separated from the rest of the body. It remained, however, for other workers actually to undertake such experiments. In 1828 Kay (4) showed that artificial perfusion with blood was capable of restoring irritability to dying muscle. Artificial perfusion of kidneys was first attempted in 1849 by Löbell (5). The work of Brown-Séquard (6) done several years later showed the necessity of oxygenation of the blood used as a perfusate. The oxygenated blood was forced through the arteries by means of a syringe. In this manner he perfused various regions including the isolated head. He found that he was able in this manner to maintain certain evidences of reflex nervous activity provided the perfusion was commenced promptly after decapitation. Ludwig and Schmidt (7) in 1868 described an apparatus by means of which blood could be forced under constant pressure from a reservoir. Improvements in aeration of the perfusion medium were made by Schröder (8). Fry and Gruber (9) devised an artificial lung by means of which the aeration of the perfusate could be accomplished without interrupting the flow of blood

to the region being perfused. Although fluctuation in the pressure supplied to the perfusion medium occurred in the work of the earlier investigators using the syringe injection method, the distinct beneficial effects of such variations in pressure were first recognized by Ludwig and Schmidt (7). Fry and Gruber (9) attached the piston of a syringe supplying the arterial pressure to a motor-driven wheel thus creating by mechanical means a pulsatile pressure. Hamel (10) emphasized the need for pulsatile pressure. He devised an apparatus in which the movements of a pendulum periodically interrupted the flow of the perfusate to the tissues, thus converting a constant pressure into an intermittent one. Jacobj (11) devised an elaborate perfusion apparatus in which pulsatile pressure was created by periodic and forcible compression of a rubber balloon placed in the arterial side of the circuit. He used the principle of aerating the blood by forcing a mixture of air and venous blood through a stretch of tubing at the end of which the blood and air were separated by gravitation. In a later paper Jacobj (12) described a method by means of which the blood was aerated by perfusion through a lung in which respiration was artificially maintained. In this manner he avoided the direct mixing of the blood with the air. In 1903 Brodie (13) published an account of an apparatus which has subsequently been used by several investigators. With it he was able to perfuse an organ with the use of no other blood than that obtained from the animal itself—a considerable advantage over many of the types previously employed. To create pulsatile pressure he suggests that a fairly distensible piece of rubber tubing placed in the arterial side be rhythmically compressed by a wooden arm.

Other forms of perfusion apparatus have been described by Hoffmann (14), Richards and Drinker (15), Friedmann (16), Mandel (17) and Kingsbury (18).

Pulse pressure as a necessary factor in the mechanics has been recently reëmphasized by Hooker (19), whose apparatus was employed in our experiments. His apparatus can be adjusted in such a manner as to furnish a pulse curve identical in form to that produced in a normal intact animal. Aeration is effected.

In addition to the purely mechanical methods of perfusion another slightly different procedure has been employed by some. As far back as 1881 Martin (20) attempted to study the activity of the heart by diverting all of the blood issuing from the aorta back into the right auricle. A heart-lung preparation was thus effected, the circulation being successfully excluded from the rest of the body. This procedure

or modifications thereof have been used by many workers since that time. In 1914 Bainbridge and Evans (21) susbtituted this living preparation for the artificial perfusion machine. The organ to be perfused received its blood directly from the aorta of the preparation. The venous blood issuing from the vein was returned to the right side of the heart. The perfused tissue thus received blood aerated by the lungs and under pulsatile pressure supplied by the heart itself. It should be remarked that in this form of perfusion the study of the perfused organ is complicated by the metabolism of the heart and lungs themselves.

EXPERIMENTAL OBSERVATIONS

All perfusion experiments were performed by use of the apparatus designed and described in detail by Hooker (19), (22). Through his courtesy we were able to obtain this machine which was made after the model of his original apparatus. We take this opportunity to acknowledge our appreciation for valuable assistance on the part of Dr. D. R. Hooker. The general experimental procedures are covered by the brief description in individual experiments. All experiments were done on dogs under complete ether anesthesia. In all perfusion experiments the dog was placed upon a warm pad to keep up the body temperature.

Perfusion of hind legs with Locke's solution

Experiment 5. Male bull pup. Weight 4.8 kilos.

Under ether anesthesia cannulae were inserted and the hind legs were perfused for ½ hour with Locke's solution. The temperature of the perfusate varied between 30° and 40°C. The perfusion pressure was between 100 and 110 mm. mercury. The pulse pressure was between 20 and 30 mm. mercury. Pulse rate 130 a minute. The 240 cc. of perfusate recovered at the end of the perfusion contained 77,000 red blood cells per cubic millimeter. Hemolysis was moderate in amount.

To test the toxicity of this perfusate the cells were removed by centrifugalization and 100 cc. of the supernatant fluid were injected intravenously into a normal dog. A rise of 1.4° in temperature with vomiting and diarrhea was noted. The pulse was not markedly altered. The perfusate was therefore moderately toxic.

Ten cubic centimeters of the centrifugalized perfusate were also injected intraperitoneally into a 100 gram rat. Slight toxicity was evident.

Perfusion of hind legs with red corpuscles suspended in Locke's solution

Experiment 7. Male collie mongrel. Weight 16 pounds.

The hind legs were perfused with a suspension of blood corpuscles in Locke's

solution. The temperature of the perfusate varied between 32° and 38°C. The mean pressure was 50 mm. mercury. The pulse rate was 130 and the rate of flow 180 cc. a minute.

Autopsy shows irregular petechial hemorrhages in the muscles and fascia of the hind legs.

Slight hemolysis was noted in the 250 cc. of perfusate recovered at the end of perfusion. One hundred and sixty-one cubic centimeters of the centrifugalized end-product were injected intravenously into a normal 13.75 pound dog. A temperature rise of 0.4° was observed. No vomiting or diarrhea occurred. Twenty cubic centimeters of this perfusate were also injected intraperitoneally into a rat weighing 100 grams. There were no evidences of toxicity from the use of this perfusate.

These two experiments (expers. 5 and 7) give little information concerning the actual perfusion conditions but supply data concerning the production of a poison by the Locke's perfusion. There is slight positive evidence for a toxic reaction in experiment 5 but a negative reaction in experiment 7. In general we have no distinctly positive evidence that this destructive perfusion of body tissues will give demonstrable evidence of a toxic element in the perfusate. As stated above this may be explained by the capacity of the body cells to remove such poisons from circulating fluids.

Perfusion below diaphragm with red corpuscles suspended in modified Locke's solution

Experiment 9. Normal female black and white pup. Weight 6.75 pounds.

The oxalated blood from a normal dog was centrifugalized and the corpuscles washed in gelatin-Locke's solution minus calcium by mixture and recentrifugalization. The red cells were then suspended in gelatin-Locke's minus calcium in the ratio of packed corpuscles 1 to solution 5. This mixture was used as the perfusion medium.

Under ether anesthesia the animal was bled. Cannulae, were inserted and the perfusate forced through the aorta and recovered from the right auricle, thus perfusing the area below the diaphragm. The temperature of the perfusate varied between 35° and 38°C. The mean pressure was maintained at 50 to 80 mm. mercury; the pulse pressure between 10 and 15 mm. mercury. The pulse rate was 130 a minute. The flow was excellent. Perfusion began 20 minutes after bleeding and was continued for 1 hour.

The autopsy was delayed for a few hours after the completion of the perfusion. The muscles of the hind legs were pale and showed very little edema. No hemorrhages were present in the muscles and connective tissue. The liver was normal except for some air bubbles in its vessels. The capsule of the kidneys stripped readily. Hyperemia was seen at the cortico-medullary boundary. Suprarenals were negative. The lymph nodes of the mesentery were normal.

The pancreas showed a considerable amount of edema. The spleen was dark red except for a transverse light band possibly caused by block from emboli. The intestines were filled with red mucoid material. The mucosa was velvety, swollen and deep purplish-red in color. Histological sections of liver and intestine give no evidence of tissue abnormality.

On centrifugalization a sample of perfusate taken 10 minutes after beginning of perfusion was light pink. A sample taken at the end of 40 minutes was somewhat deeper in color, while a sample taken at the end of perfusion was dark red.

One hundred and fifty cubic centimeters of a centrifugalized sample taken after 10 minutes of perfusion were injected into a small normal dog. The animal vomited once. There was a rise of 1°C. in temperature. The pulse remained good. No marked symptoms of intoxication were present.

Eight cubic centimeters of the 10-minute sample were injected intraperitoneally and 2 cc. were injected subcutaneously into a 75-gram rat. No toxic action was noted. This test was repeated by injecting 5 cc. intraperitoneally into a 38-gram rat. No toxic action was noted. The same amount of the 40-minute perfusate sample was injected intraperitoneally into a 62-gram rat. No toxic action.

Perfusion below diaphragm with red blood cells in modified Locke's solution

Experiment 11. Female shepherd pup. Weight 2280 grams.

In preparation of the perfusate blood corpuscles were obtained from the blood of a normal dog bled several hours previously into a 1 per cent sodium oxalate solution. The red cells were centrifugalized and washed in Rous' gelatin-Locke's solution minus calcium. The packed cells were then suspended in a similarly prepared calcium-free gelatin-Locke's solution in the ratio of one part of corpuscles to five of the saline mixture.

Under ether anesthesia the animal was bled and the cannulae were arranged to perfuse all of the tissues below the diaphragm. Ten minutes were consumed in arranging the cannulae. The temperature of the perfusate was between 35° and 38°C. The pulse pressure was about 10 mm. mercury. Due to clots in the gauze two stops were necessitated over a period of 5 minutes each. One occurred soon after the beginning of perfusion and one some minutes later. The duration of the perfusion was 1 hour. During this period the animal increased 760 grams in weight.

Autopsy showed about 75 cc. of pale bloody fluid in the abdominal cavity. Marked edema was present about the pancreas and throughout the mesentery. Hemorrhagic spots were observed over the surface of the kidney and stomach, about the ovary and throughout the muscles and fascia of the hind legs. The liver on section was translucent. On section the kidney showed indefinite dark hemorrhagic spots up to 0.5 cm. in diameter. The whole organ was dark and congested. The stomach contents were normal. Mucus and bloody fluid were present in the small intestine. Congestion and bloody intestinal contents were more prominent in the lower part of the small intestine.

The perfusate showed a moderate grade of hemolysis before perfusion but less after 15 minutes of perfusion. Moderate hemolysis existed at the end of the perfusion.

Bacteriological cultures showed 500 bacteria per cubic centimeter in samples taken at end of perfusion.

Two hundred and eight cubic centimeters of the perfusion fluid obtained at the end of the perfusion were injected intravenously into a normal small dog. With the exception of a temperature rise of 1.5° and some shivering there were no signs of intoxication.

The two experiments (expers. 9 and 11) show the results of a perfusion of all the organs and tissues below the diaphragm by a red cell Locke's solution mixture. It is to be noted especially that there is marked edema of retroperitoneal tissues and the pancreas. This edema is invariably present in considerable amount except when whole defibrinated blood is used as perfusate. We accept the edema as one indication of tissue or cell injury. The same is true of hemorrhagic areas and ecchymoses, but some of them may be due to emboli. The marked congestion of the intestinal mucosa with the escape of blood-tinged fluid and mucus is also a valuable index of injury. This is a familiar reaction noted in dogs dead from anaphylaxis or large doses of proteose or from surgical shock. The perfusate in both these experiments contained no poisonous substance for normal dogs and white rats.

Hemolysis is always present in slight or moderate degree in all our experiments. We are inclined to explain a part of this hemolysis by the cell injury in organs or tissues and this cell injury reacts unfavorably upon the red cells with resulting hemolysis. We realize that the dog's red corpuscles are most fragile and that the red cells are subjected to much mechanical injury in these experiments. Other observers may choose to explain all this hemolysis upon a purely traumatic basis.

Perfusion below diaphragm with diluted defibrinated blood

Experiment 13. Male bull pup. Weight 2150 grams.

Two parts of defibrinated blood obtained from a normal dog were diluted with one part of gelatin-Locke's solution. The animal was anesthetized with ether, the thorax opened and the cannulae inserted in such a way that the perfusion medium was forced into the aorta just above the diaphragm and the blood received from the inferior vena cava just below the heart. In this way the entire region below the diaphragm was perfused. The temperature of the perfusion medium was maintained at about 32° to 38°C. The systolic pressure varied from 95 to 120 mm. mercury with a pulse pressure of 20 mm. mercury. The return flow was accidentally occluded for a few seconds at the beginning of the experiment. The perfusion lasted 1 hour.

Examination of the region perfused showed hemorrhagic streaks in the diaphragm and gall bladder. Numerous small areas of hemorrhage accompanied by a considerable amount of edema existed about both kidneys. The liver showed considerable congestion and edema. The lobules of the pancreas were

distinctly separated by edema. The spleen was quite dark. The stomach showed considerable engorgement with sub-mucous hemorrhages. Externally the small intestines were spotted by numerous small subserous hemorrhages. The intestinal mucosa showed diffuse congestion while the lumen contained a little dark mucus. The colon was more nearly normal in appearance; however, a few small sub-serous hemorrhages were seen. The hind legs showed no hemorrhage or edema. There was a weight increase of 400 grams.

The perfusate showed slight hemolysis before perfusion and a moderate grade of hemolysis at the end of perfusion. The perfusate obtained at the end of perfusion contained 39 mgm. of non-protein nitrogen and 16 mgm. of urea-nitrogen per 100 cc.

TABLE 34

Perfusion below the diaphragm with defibrinated blood. Experiment 16

TIME	COLOR OF CENTRIFUGALIZED PERFUSATE	CARBON DIOXIDE CAPACITY PER 100 CC.	HYDROGEN ION CONCENTRATION	REMARKS
Before	Pale pink	38.5	7.5	
After 20 minutes	Rose	15.7	7.3	
After 40 minutes	Rose	26.1		More blood added
At the end	Deep rose	12.8	7.4	

Perfusion below the diaphragm with defibrinated blood

Experiment 16. Normal male bull pup. Weight 4.2 pounds.

Under ether anesthesia cannulae were inserted for perfusion below the diaphragm. This region was perfused for 30 minutes with pure defibrinated blood at a rate of 100 cc. per minute. The cannulae were then shifted to the lower abdominal vessels and the hind legs perfused at a rate of 25 cc. a minute for 60 minutes, with the same perfusate. The perfusate was aerated with pure oxygen and was maintained at a temperature varying from 36° to 39°C. The pulse rate was 141 a minute.

Intestinal peristalsis was quite conspicuous at the beginning of perfusion but was less noticeable after the perfusion had been in progress for about 5 minutes. During this period the abdominal wall was very sensitive to touch and contracted violently when touched.

At autopsy a large amount of clear straw-colored fluid was noted in the abdominal cavity. The animal had gained 200 grams in weight as a result of the perfusion. The liver was slightly translucent although apparently normal. The spleen was somewhat congested. The pancreas showed no edema. The kidneys were slightly congested in the pyramidal areas. The stomach was normal. The duodenum was likewise normal but the ileum showed a mucosa congested and dark red with grey mucoid material in the lumen. The hind legs showed no edema and were quite dry in appearance. A few petechial hemorrhages appeared in the fascia.

Histological sections: Pancreas and kidney are normal. The spleen and liver show capillary congestion but normal parenchyma cells. The stomach and small intestine are normal except for slight congestion of the ileum.

In these two experiments (expers. 13 and 16) we used diluted or whole defibrinated blood. The general autopsy picture following the use of whole blood is almost normal and the lack of the edema we believe is to be explained on this ground. Even in the last experiment we note the development of ascites which of course indicates circulatory abnormality. There is further a distinct acidosis to be explained by inadequate aeration of the blood.

Perfusion with whole defibrinated blood—toxic proteose added

Experiment 17. Adult male poodle dog. Weight 10.25 pounds.

Under ether anesthesia the animal was bled. The arterial cannula was placed just above the bifurcation of the iliacs. The venous cannula was inserted in the inferior vena cava just above the renal veins. Thirty minutes were consumed in bleeding, arranging the cannula and starting the perfusion flow. The hind legs were perfused for 32 minutes with defibrinated blood. To the 500 cc. of perfusate then remaining in the apparatus 100 cc. of a *proteose solution* (lethal dose is 2 cc. per pound body weight, adult dog) were added and the perfusion continued for 12 minutes. The perfusate was warmed to a temperature of 35° to 38°C. The rate of flow was 80 cc. per minute until near the end of the experiment, when it decreased to 50 cc. per minute. The aeration of the perfusate was excellent. The proteose solution was prepared as described elsewhere (23) from the material of the obstructed intestine.

Autopsy of the perfused dog showed slight icterus of the tissues of the hind legs but no hemorrhages or edema. A few small hemorrhages were found in the right testicle. The pelvic organs were negative.

At the end of perfusion there were obtained 300 cc. of perfusate for analysis. Of this were injected into a 14-pound normal pup, 227 cc., which represented 38 cc. of the original proteose. This would be a fatal dose for an adult dog weighing 19 pounds, provided no proteose had been lost. Pups are more susceptible to proteose intoxication than adults. This dog therefore received a theoretical dose of one and one-half times its lethal dose, assuming that no proteose was lost from the perfusate during the perfusion.

The injection of 227 cc. of perfusate caused death in 3 hours with the clinical picture of acute proteose intoxication. Diarrhea and vomiting appeared within ½ hour after injection and continued until death. There was an initial rise in temperature followed by a drop to 36.8°C. half an hour before death. Autopsy findings showed exquisite splanchnic engorgement especially marked in the mucosa of the small intestines, which was a velvety purplish-red coated with mucus—described and pictured elsewhere (24).

The results of examination of the perfusate are given in the table below (table 35).

Perfusion with whole defibrinated blood—toxic proteose added

Experiment 18. Under ether anesthesia a small female mongrel dog was bled. Cannulae were inserted preparatory to perfusing all of the body below the diaphragm. Defibrinated blood warmed to 35° was perfused through this region under a mean pressure of 110 mm. of mercury. The pulse pressure varied between 5 and 15 mm. of mercury. The pulse rate was 120 per minute. Aeration of the perfusate was not quite as satisfactory as usual. Actual perfusion commenced 30 minutes after bleeding the animal. After the flow has been maintained for 20 minutes 100 cc. of proteose solution were added to the 250 cc. of defibrinated blood then remaining in the apparatus. Perfusion was continued for 15 minutes. The rate of flow was 150 cc. a minute at first, but gradually decreased to 85 cc. a minute toward the end of the experiment. Marked intestinal peristalsis was present during the first few minutes of perfusion; blood-tinged feces later.

Autopsy showed a moderate quantity of pale blood-tinged fluid in the peritoneal cavity. The animal had gained 180 grams in weight during the experiment. The liver showed edema and small hemorrhages throughout. Numerous small hemorrhages as well as several larger ones were seen in the wall of the gall bladder. The pancreas was negative except for one small hemorrhage. The mesenteric lymphatics contained blood-stained fluid. The adrenals contained several small hemorrhagic areas. The kidneys were negative. The spleen was small, dark and translucent. The stomach showed one fairly large but no small hemorrhages. The mucosa of the duodenum was engorged. The intestinal lumen contained an excess of thin bloody fluid with little mucus. It is possible that some of the hemorrhages noted above are to be explained by short periods of high blood pressure during the periods of perfusion.

As was stated above, 100 cc. of the proteose solution (lethal dose is 2 cc. per pound body weight, adult dog) were added to the 250 cc. of perfusate then in circulation and the perfusion continued for 15 minutes. Of the final perfusate 77 cc. were injected intravenously into a normal 11-pound pup (no. 17–181). It is evident that these 77 cc. contained 22 cc. of the original proteose solution provided none of this toxic material had been removed during perfusion by the tissues of the animal perfused. The reaction of the animal might be expected therefore to be lethal, if no proteose was removed during the perfusion through the tissues of the first dog. The reaction to this intravenous injection was typical for a *sublethal* toxic dose of proteose. There was vomiting and diarrhea for 2 hours and much prostration. Recovery was evident in 3 hours and the dog was normal in a few more hours. It appears that some of the proteose had been removed as the amount given was more than a lethal dose for a pup of 11 pounds body weight.

The final perfusate was further examined and shown to contain definite amounts of hemoglobin (hemolysis). Bacteriological examination (plates) showed 40,000 colonies per cubic centimeter of the perfusate. The non-protein nitrogen at beginning of perfusion was 34.7 mgm. per 100 cc. of perfusate and at the end was 35.8 mgm.

In these two experiments (expers. 17 and 18) the perfusion was done with whole defibrinated blood to insure a minimum injury of the perfused tissues. After the initial perfusion a standardized toxic solution was added to the perfusate and again circulated as before. The perfusate at the end of the experiment was tested on a normal dog and in this way it was demonstrated that some of the poison had been removed. It is easy to show that large doses of this toxic proteose are rapidly removed from the circulation of a dog. Following intravenous injection of large amounts of toxic proteose it is possible to demonstrate its presence for 2 to 3 minutes in the blood stream but not after 5 minutes. The presence of bacteria as noted in this and other experiments will not seriously disturb the reaction. If anything, their presence will increase the toxicity of the perfusate mixture. These bacteria probably gain

TABLE 35

Perfusion with whole defibrinated blood—toxic proteose added. Experiment 17

TIME	HEMOLYSIS	PER CENT BLOOD CELLS	UREA NITROGEN	NON-PROTEIN NITROGEN	HYDROGEN ION	CARBON DIOXIDE CAPACITY
			mgm. per 100 cc.	*mgm. per 100 cc.*		
Before perfusion.....	Moderate	47	19	26	7.5	16
After one-half hour....	Moderate	50	16	31	7.4	16
On adding proteose....	Moderate	47	18	30	7.4	19
At end of perfusion....	Slight	45	20	89	7.4	13

entrance in part through the intestinal tract and in part are added by the manipulation of the perfusate. Efforts were made to preserve the circulating machinery in as near a sterile condition as possible but there are many possibilities for introducing contamination.

The "proteose solution" used in these experiments is prepared from material obtained from obstructed intestines or closed intestinal loops in dogs. The material is precipitated by five volumes of alcohol, the precipitate dissolved in water and the protein removed by boiling in dilute acetic acid solution. A second precipitation with alcohol is often employed. The final solution is an opalescent fluid which contains proteose-like materials. This fluid material is then standardized by intravenous injection in normal dogs, as has been described elsewhere (23).

Large infusion of Locke's solution into portal vein

Experiment 21. Dog 17–125. Normal adult mongrel, black and tan. Weight 43 pounds.

Under ether anesthesia and with sterile precautions the abdomen was opened and the hepatic-pancreatico-duodenal and pancreatico-duodenal arteries were ligated. Sterilized calcium-free Locke's solution, 1750 cc., was warmed to approximately 36°C. and injected at a rate of 55 cc. a minute into the portal system through a small venous branch in the mesentery. The abdomen was closed. The animal showed no severe reaction for some time but died 3 days later.

Autopsy performed several hours later shows considerable post-mortem change. Dark red softened areas containing bubbles of gas are seen scattered throughout the liver. The serosa of the intestines is quite red. The mucosa shows moderate engorgement and is covered by a buttery exudate. The coil of intestine to which the perfused vein was distributed differs in no way from the other parts of the small intestine. Kidneys are negative. The total non-protein nitrogen of the blood shows but slight alteration from the original value as a result of the experimental procedure.

Histological sections: The picture is somewhat confused by post-mortem changes but it is clear that there are scattered areas of liver cell necrosis which are ante-mortem and presumably related to the experimental procedure. These areas include many liver lobules and present a uniform necrosis with scattered leucocytes between the liver cell strands. Bile ducts and blood vessels are normal and no evidence of vascular thrombosis is observed in any sections. Other sections show normal liver parenchyma. Other tissues present nothing of importance.

Large infusion of Locke's solution into splenic artery

Experiment 23. Dog 17–200. Young male collie. Weight 29.5 pounds. Normal except for a slight attack of distemper.

Under ether anesthesia and with sterile precautions the abdomen was opened. A cannula was inserted into the splenic artery in such a manner that the upper arm of the pancreas, a part of the duodenum and stomach, as well as the liver, were perfused by 2000 cc. of sterile calcium-free Locke's solution injected at a rate of 50 cc. a minute. The saline was injected at room temperature. Splenectomy followed the perfusion. The pancreas showed moderate edema at the end of the experiment, but the dog was not severely shocked. Later a state of intoxication slowly developed and at the end of 36 hours death was imminent. The animal was killed by ether.

Autopsy performed at once shows a little blood-stained peritoneal fluid. The liver shows a moderate grade of cloudy swelling. The subserous tissues of the gall bladder are thick and edematous. A number of sub-serous ecchymoses are scattered over the small intestine. Several hemorrhagic areas are present in the mucosa of the small intestine. Hemorrhages and fat necrosis are rather pronounced in the upper arm of the pancreas.

Histological selections: Organs are normal with the exception of the pancreas and liver. The pancreas shows extensive hemorrhagic necrosis and much nec-

rosis of fat and gland parenchyma. There are no thrombus masses noted in any of the vessels. The head of the pancreas is essentially normal. The liver shows scattered clumps of polymorphonuclear leucocytes and evidence of injury to small clusters of liver cells in various portions of the liver lobules.

Large infusion of Locke's solution into the portal vein

Experiment 24. Dog 17-203. Adult male mongrel. Weight 29.5 pounds. Slight distemper.

Under ether anesthesia the abdomen was opened and the hepatic artery clamped. One of the larger splenic veins was isolated, a cannula inserted and 1500 cc. of sterile calcium-free Locke's solution warmed approximately to body temperature were injected into the portal system over a period of 18 minutes. The clamps were then removed from the hepatic artery. Splenectomy was performed and the abdomen was closed. The temperature rose to 40°C. shortly after the operation and the animal vomited once; otherwise no clinical disturbance was noted. Complete recovery ensued. The animal was killed 7 days later. The autopsy was negative. No alteration could be made out in the liver.

To obviate the mechanical difficulties inherent in organ perfusion and to get information concerning the direct effect of Locke's solution upon tissue and organ cells we performed a number of experiments of which experiments 21, 23 and 24 are examples. The first experiment (exper. 21) shows a fatal reaction following a large infusion in the portal vein after ligation of the branches of the hepatic artery to limit the blood flow through the liver. We have explained this reaction as due in part to injury of the liver cells by contact with the mixtures of Locke's solution and blood. It is known that ligation of the hepatic artery will cause no disturbance in the dog. It seems hard to account for these areas of liver necrosis except as due to the action of the portal blood diluted by the large infusion of Locke's solution into the portal vein. It is to be noted (exper. 24) that a similar experiment was tolerated without obvious liver injury but the occlusion of the hepatic artery in this experiment was only temporary.

The pancreas necrosis was surely caused by the perfusion of the splenic artery (exper. 23). It may be objected that perfusion in this way against the arterial stream will cut off the tissues from oxygen by washing away all available red cells. We are inclined to believe that arterial collaterals which are numerous in this region will insure the presence of the necessary number of oxygen-carrying red cells. This objection cannot apply to experiment 21.

DISCUSSION

In attempting an analysis of our own experiments we wish to draw freely on the published work of other investigators. We wish to keep constantly in the reader's mind that *physiological perfusion* of any organ is a matter of extreme difficulty and often great confusion is introduced by such methods which are intended to simplify the study of organ function. By *physiological perfusion* we mean a perfusion adequate to maintain the organ in its normal physiological activity.

In the first place let us inquire what criteria of tissue abnormality we have. What sort of evidence is going to lead us to pass judgment concerning the physiological condition of tissues? There are certain conditions under which we may ascertain what is going on in the tissues by a direct observation of the functional activity of the part perfused. For example, in perfusing the kidney the quantity and quality of the urine secreted furnishes some evidence concerning the condition of that organ. The reduction within the organ of oxyhemoglobin to hemoglobin was noted by the earliest observers and is indicative of metabolic activity of some nature on the part of the perfused tissues. The nature of the heart beat is indicative of the condition of the perfused heart, although Magnus (25) has shown that if such an inert substance as hydrogen gas be perfused through the coronary arteries heart beats will be stimulated. Sollmann (26) showed that the same result followed perfusion with cottonseed or paraffin oil. In such cases the perfusion fluid cannot be thought of as being a nutrient fluid; on the other hand it is not altogether impossible that, as was suggested by Sollmann, the heart beats may be stimulated by purely mechanical factors. For these reasons we must not hastily conclude that, because the gross mechanical movements simulate those occurring in the intact animal, the preparation is in fact an example of normal physiological activity.

A criterion as to the condition of the perfused medulla is furnished by observing whether the medulla continues to maintain its normal control over the heart and muscles of respiration.

In addition to direct observation of the functional activity of an organ perfused, we have still other kinds of evidence which help us to judge concerning the condition of the tissue. Thus the rate of flow through the vessels is in some cases a valuable indicator for it is a general rule that tissue injury brings about in some way or other a decrease in the rate of flow through the part. We have, in addition, the still more crude signs of tissue injury: edema, congestion and hemorrhage.

Of the factors in the procedure of perfusion whose variations might bring about injury to the part, the following may be mentioned as being perhaps the most important: aeration of the perfusion medium, composition of the perfusion medium, interruptions in continuity of flow, temperature of the perfusion medium when it enters the perfused organ, mean pressure and pulse pressure.

It should be realized that a perfusion experiment is no better than its weakest point. If any of the above factors react in such a way as to cause injury to an organ, perfection of the other factors will not remedy the defect. It is also conceivable that when several organs are being perfused simultaneously, injury to one organ or tissue may react injuriously on others.

Concerning the effect on the tissues of composition of the perfusate, the literature contains many references to condition of the tissues as shown by functional activity. The injurious effects of foreign blood have been known since the time of Prévost and Dumas (27) when this fact first began to be recognized through the failure of foreign blood to act normally after transfusion. Though repeatedly shown to be harmful in its effects on tissues of another species, foreign blood has been used in perfusions even as late as Brodie (13) who says that ox, sheep or horse blood cannot be used in the perfusion of organs taken from dogs. He finds that as soon as foreign blood is supplied to the perfused heart the beat becomes irregular. The heart next goes into fibrillary twitchings and cannot be recovered from this state even with the animal's own blood.

Although defibrinated blood had been used in the transfusion experiments of Prévost and Dumas (27) without the observation of harmful results, Magendie found it incapable of carrying on the normal function of the circulating medium after transfusion. In a series of experiments in 1822 (28) and again in 1838 (29) he presented experimental data to show that the lack of fibrin, reduced through repeated bleeding, defibrination and reinjection of the defibrinated blood gives rise to a serous and bloody transudate into the lungs and intestine with the death of the animal.

The weight of Magendie's name behind such a statement did much to discredit defibrination in the eyes of other workers, but eventually Bischoff (30), Goll (31), Polli (32), Panum (33), Ponfick (34), and many others began to turn the weight of experimental evidence against a belief in the extreme toxicity of defibrinated blood when used in transfusions between animals of the same species

More recently, by means of perfusion experiments, Stevens and Lee (35) and Brodie (13) present evidence of slight vasoconstriction due to the use of defibrinated blood as a perfusion medium. Their work again points to an injurious action of defibrinated blood when substituted for the normal circulating medium. However, Stevens and Lee believe that the slight vascular contraction which they note can be readily counteracted with pharmacological agents.

In 1903 Pfaff and Vejux-Tyrode (36) found defibrinated blood definitely injurious to the kidney of the dog. Quantities of from $\frac{1}{7}$ to $\frac{1}{10}$ of the total blood were withdrawn from the carotid artery, whipped, filtered and reinjected into the jugular vein. The repetition of this procedure resulted in the appearance of albumin, hemoglobin and red blood cells in the urine and finally cessation of secretion. However, a rapid return to normal was effected in these animals by bleeding followed by direct transfusion of whole blood from a normal dog. It would seem that the kidney may be unusually sensitive to this procedure.

It is certain that in those of our animals which underwent quite complete defibrination (37)—(see expers. 323 and 324)—there were no clinically evidenced signs of injury or toxic manifestations. This may also be said of those experiments of Whipple and Goodpasture (38) in which quite complete defibrination was also effected.

The importance of a physiological balance of the normal inorganic salts of the blood is generally recognized. Solutions containing abnormal quantities of these salts have been shown to be toxic to the perfused heart. Hooker (22), (39) showed that in perfusion of the respiratory center a balance of potassium and calcium is essential for a normal function of the preparation. As was early shown by several investigators and more recently reëmphasized by Hooker (19) and by others, and as we have found in our experiments, saline solution has the property of setting up such a condition in the tissues that the rate of flow gradually decreases.

The effects of variations in composition of perfusion media may also be manifest from the morphological side. Brodie (13) shows that edema results from the use of foreign blood. Hamel (10) shows that edema results in organs perfused with saline under pulsatile pressure. Similarly the kidney when perfused with Locke's solution exhibits more edema then when perfused with defibrinated blood (19). These results are entirely in accord with our experience. Perfusion with pure Locke's solution almost invariably produces an extreme grade of edema. Dog's defibrinated blood diluted with Locke's solution produced less,

and pure defibrinated blood produces very little demonstrable edema. It is difficult to explain just why dilution with Locke's solution should produce edema. In several of our experiments we carried out hydrogen-ion and carbon dioxide determinations on the perfusate and it is interesting to note that in cases of marked edema there was a rise in the hydrogen ion concentration and a fall in the carbon dioxide capacity. Whether the edema is the result of the acidosis or not, there still remains the question as to what is the cause of the decrease in the buffer substances. Perhaps it may be attributable in part at least to insufficient oxidation in the tissues. Poor oxidation may result from insufficient oxygen-carrying capacity of the perfusate or from inadequate aeration of the perfusate in the artificial lung, or may result from a decreased rate of flow through the animal and a stagnation of the blood in the tissues with consequent asphyxia.

A factor which has been shown to be of considerable importance in causing tissue injury is that of loss of time in establishing the artificial perfusion after interrupting the normal relations. Most of the earlier workers paid but scant attention to this phase of the problem. In many experiments several hours elapsed before any attempt was made to reëstablish the flow. Grube (40) mentions that in perfusion of the liver with defibrinated blood to which glucose had been added, the glycogen content of the liver rises, but only in case the circulation is very promptly reëstablished. The deleterious effect on the kidney of temporary anemia is well known. Momentary compression of the renal vessels may cause a cessation of secretion for many minutes. The effect of compression of the cerebral vessels has been known since very early times. The duration of such anemia necessary to produce irreparable damage was long ago studied by Astley Cooper (41). Signs of activity can be restored to the brain of the isolated head provided only that the perfusion is promptly commenced (6), (42), (43) and (44). Skeletal muscle is capable of surviving much longer periods of anemia than is the case of brain or kidney. Munk (45) holds that in perfusion of the kidney if the flow is not promptly commenced the vessels become narrowed, the perfusion flow rendered difficult and a delay in the formation and flow of urinary fluid occurs. Recently Bainbridge and Evans (21) have succeeded in perfusing the kidney without any interruption whatsoever in the continuity of flow.

A great deal has been said in the literature concerning the value of pulsatile pressure. As has already been noted, Ludwig and Schmidt (7) observed that with constant pressure the rate of outflow from the

perfused tissue decreases, but that recovery occurs if the flow is halted for a while. Similar observations were subsequently made by many workers. The recognition of the importance of this factor is evidenced by the numerous forms of apparatus devised to accomplish this end. Hooker (19) holds that in the perfused kidney the amount of urinary filtrate formed varies directly with the magnitude of the pulse pressure. The amount of proteins in the urinary filtrate varies inversely with the magnitude of the pulse pressure. The rate of blood flow through the organ varies directly with the magnitude of the pulse pressure. Recently Gesell (46) has been able in the intact animal to abolish almost completely the pulse pressure in the renal arteries without interfering with the normal mean blood pressure. The result of this alteration was an immediate and practically complete cessation of urinary secretion.

SUMMARY

Physiological perfusion of organs is a matter of great difficulty. Much of the work done with organ perfusion is of little value because a proper appreciation of the limitations of the method does not obtain among laboratory workers.

The use of physiological saline, Locke's solution or various modified solutions with or without red blood corpuscles does not permit of *physiological perfusion* of organs. The contact of these salt solutions with the tissue cells will result in profound injury or actual cell destruction. Any deduction made from experiments of this nature must be limited by these facts just outlined.

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THE SEASONAL VARIATION IN THE GROWTH OF BOSTON SCHOOL CHILDREN

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I

In 1892 I secured the measurement of weight and height, and other physical dimensions, from 34,500 boys and girls in the public schools of St. Louis. In this investigation, as in similar studies upon which our present standards are chiefly based, all the measurements were collected at one time, once for all. The children were then distributed by sex and age, and the median weight and height, etc., determined for each year. This is the "generalizing" method. Its economies are great. Thus, in the St. Louis investigation, a little more than a million data were harvested in eleven weeks, and work in the class room was interrupted for only seven half-hour periods.

Far different is the "individualizing" method. This procedure demands the measurement of the same child again and again, throughout its period of growth. The measurements must begin at the earliest age with many thousand children, lest death and desertion so thin the ranks that the survivors will be too few for safe statistical treatment. The individualizing method demands, therefore, a formidable expenditure in time and effort through many years. Toilsome as this task may be, it cannot be foregone. For the generalizing method conceals a grave flaw; it does not give the growth of the individual child.[1]

With this in mind, I asked the Boston School Committee, in 1909, to measure the height and weight of several thousand of the youngest children and to repeat the measurements monthly in the same children throughout their school life. The measurements were made by the school nurses, under the direction of Dr. T. F. Harrington, and after his lamented death they were continued by his successor, Dr. W. H.

[1] This defect in the generalizing method and the contrasting advantages of the individualizing method were discussed by me in the Quarterly Publications of the American Statistical Association, December, 1893.

Devine. In this land of criticism, in which our minds are often occupied with the real, and still more often with the imaginary defects of our public institutions, the patient, laborious collection of these measurements, month after month, year after year, is a monument of devotion in which we may all take pride.

II

These measurements of the weights and heights of Boston children throughout their school life were completed in June, 1919. The statistical analysis began the following month. The first step was to distribute the weights according to age in months. For example, all boys 110 months of age were placed in one group, and the median weight for that group was calculated. It will be observed that the principle of this first distribution is still that of the generalizing method. This principle must be clearly apprehended. In the generalizing method the individual measurements are distributed into groups, by which distribution the personal character of each individual is lost—the individual becomes merely a statistical unit. Whether the measurements so distributed have been made monthly, or yearly, or whether all the children are measured but once and the measurements distributed by years of age, is immaterial; the essential mark of the generalizing method is the loss of personal identity—the transformation of the boy or girl into a statistical unit.

In this present investigation the first distribution of boys' weights, described above, is therefore a distribution by the generalizing method. Its fruits are given in table 1, which records the median weight of boys at each month of age from the 60th to the 176th month, inclusive. In this table, the months are not calendar months, but months of age. For example, the weight opposite 110 months is the median weight of 1226 boys born in 1904, 1905 and 1906. Of these, 33 were born in October, 1904; 71 in January, 1905; and 10 in April, 1906. The October boys reached 110 months of age in December, 1913; the January boys in March, 1914; and the April boys in June, 1915.

When the data in table 1 are plotted, there results the curve shown in figure 1. This curve of monthly growth is in principle that also obtained when the median value is calculated for each year, and these annual values are connected by a line—the curve of growth, old style. These curves rise steadily and smoothly in an unbroken line.

Such curves have given rise to much loose thought; for a curve obtained by the generalizing method is a statistical and not a personal

TABLE 1

Weights at each month of age from 60 through 176. Boys born in 1904, 1905 and 1906

MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS
60	42.75	72	44.00	84	47.68	96	52.48	108	57.74
1	41.10	3	44.25	85	48.30	7	52.96	9	58.34
2	41.30	4	44.35	6	48.78	8	53.74	110	58.64
3	42.00	75	45.22	7	49.29	9	53.90	1	59.51
4	41.44	6	44.97	8	49.80	100	54.41	2	60.01
65	42.26	7	45.65	9	50.09	1	54.91	3	60.54
6	42.31	78	45.67	90	50.56	2	55.27	4	60.94
7	42.06	9	46.09	1	50.73	3	55.87	115	61.58
8	41.98	80	46.34	2	51.25	4	55.83	6	61.56
9	42.56	1	47.03	3	51.32	105	56.27	7	61.83
70	43.17	2	47.18	4	51.85	6	56.76	8	62.36
1	44.53	3	47.47	95	52.22	7	57.23	9	62.95
120	63.42	132	69.42	144	75.21	156	82.19	168	92.75
1	64.22	3	70.17	145	76.23	7	83.56	9	93.13
2	64.96	4	70.90	6	76.44	8	84.43	170	93.88
3	65.14	135	71.60	7	77.48	9	85.94	1	97.00
4	65.69	6	72.85	8	78.17	160	86.66	2	92.67
125	66.46	7	72.92	9	78.55	1	87.56	3	94.00
6	66.97	8	73.13	150	79.82	2	88.47	4	96.50
7	67.49	9	73.67	1	80.24	3	89.44	175	96.00
8	67.67	140	73.72	2	80.22	4	90.17	6	95.50
9	68.31	1	74.50	3	80.96	165	91.58		
130	68.49	2	74.43	4	80.98	6	92.25		
1	68.97	3	74.90	155	81.53	7	91.19		

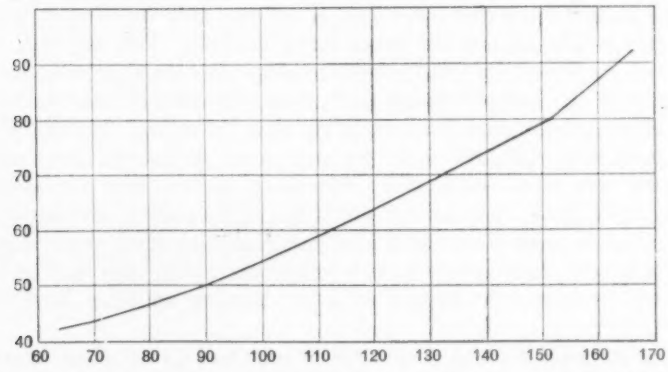

Fig. 1. The median weight of boys at each month of age from the 60th to the 176th month, inclusive (table 1). In this figure the months are not calendar months but months of age. The ordinates are pounds.

phenomenon. This will be clear, if we consider the personal history of two boys, whom we will call John and James. At age six, John is 30 per cent above, and James is 30 per cent below the average. But John's family fall upon evil days; illness and poverty pursue them. The family of his comrade prosper; James spends his vacations in the country, where he has a horse to ride; fresh air and good food do their accustomed work. At age sixteen, James and John have exchanged places; James has risen to 30 per cent above the average and John has fallen to 30 per cent below it. Their personal fortunes have altered; but they are still the same distance from the average. The inhuman average is unmoved by the deplorable fate of John and the happy success of James—to the statistical average, John and James are not persons, but statistical units. Thus the generalizing method *per se* gives no accurate information as to the growth of the individual child. Nor can this defect be removed by measuring repeatedly the same children throughout their period of growth, either monthly or at any other interval, so long as the resulting measurements are treated as statistical units. Thus, table 1 and figure 1 give accurately the monthly increase in the statistical median value, and they give with accuracy the relation between the size of any individual child and the size of other children of the same age; but they do not give, with certainty, the increase in weight of any child. They are not standards of growth, but merely standards of relative size.

III

The extinction of the individual is not the only indictment which can be brought against the generalizing method. This method, still so much employed, is blind to the possibility of seasonal growth.

Consider the case of Hyman Katz, drawn at random from the mass of children whose growth histories are now before us. Hyman Katz was born in August, 1905. In his ninth year, he gained four pounds between September and January, whereas he did not gain any weight, to speak of, between February and June. The case of Hyman Katz is the starting point of a series of interesting and important observations. It will be seen that Hyman Katz is not an exception, save that he is a somewhat extreme illustration of a law binding on other boys and girls.

Examine table 3 which deals with all boys and girls born in August, 1905. Again the growth in weight is much larger in the second half

TABLE 2

Growth in pounds of Hyman Katz 1914–15

MONTH	WEIGHT	GAIN IN WEIGHT	MONTH	WEIGHT	GAIN IN WEIGHT
1914	*pounds*	*pounds*	*1915*	*pounds*	*pounds*
September	56.50		January	60.50	0.37
October	57.00	0.50	February	60.50	0
November	58.25	1.25	March	60.00	−0.50
December	60.13	1.88	April	60.00	0
			May	60.50	0.50
			June	61.00	0.50

TABLE 3

Gain in weight of boys and girls born in August, 1905

YEAR	BOYS		GIRLS	
	September to January	February to June	September to January	February to June
	pounds	*pounds*	*pounds*	*pounds*
1912–13	3.34	+0.75	1.88	0.12
1913–14	2.17	−0.17	2.50	0.65
1914–15	2.85	+0.67	2.96	0.13
1915–16	1.29	+0.81	1.63	0.71
1917–18	5.85	−0.07	3.19	0.81
1918–19	4.88	+2.90	4.63	1.83
Average.....	3.40	0.82	2.79	0.71
Ratio.....	$\frac{3.40}{0.82} = \frac{4.1}{1}$		$\frac{2.79}{0.71} = \frac{3.9}{1}$	

The year 1916–17 is omitted because the schools were closed in September, 1916, on account of an epidemic.

of the year than in the first half. This conclusion is supported by the examination of the weights of boys born in all the months of 1905, when these weights are distributed by the months of the year. In table 4 the median weight of all boys born in 1905 is given for each month of the year, and in table 5, the increase in the median weight is recorded. Seasonal growth is again demonstrated. The data in table 4 are reproduced in figure 2.

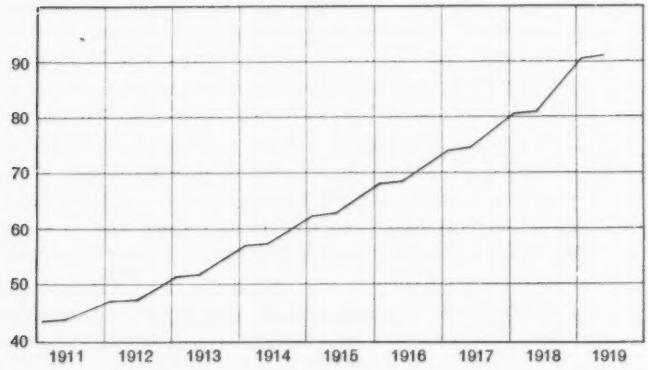

Fig. 2. The weights of boys born in 1905, distributed by months of the year. The ordinates are pounds. (See table 4.) Fig. 2 is the true curve of growth in weight; it shows the seasonal variations. Compare this figure with Fig. 1, the curve of growth "old style," in which the seasonal variations are lost.

We have dealt thus far with the absolute increase in weight. It is desirable to examine also the percentile increase, i.e., the absolute increase of each month divided by the weight at the beginning of the month. As would be expected, the seasonal variation again appears. The average total percentile gain in the first five months of the years 1913, 1914, 1917 and 1918 is 1.89; whereas in the last five months of those years, the average total percentile gain is 6.61.

TABLE 4

The weights of boys born in 1905, distributed by months of the year

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1911	43.00	42.23	42.93	42.83	42.48	42.46	46.20	45.04	45.21	45.31
1912	46.82	46.70	46.62	47.03	46.99	47.03	48.98	49.22	49.98	50.38
1913	50.94	51.49	51.73	52.18	52.05	51.97	53.31	54.16	55.00	55.37
1914	56.45	56.73	57.19	57.38	56.99	56.82	58.34	59.38	60.05	60.79
1915	61.50	62.08	62.38	62.48	62.50	62.72	64.94	65.02	65.66	66.47
1916	67.09	67.88	68.34	68.58	68.32	68.53		71.50	72.18	72.89
1917	73.39	73.81	74.21	74.43	74.81	74.79	76.49	77.32	78.27	79.23
1918	80.17	79.88	81.13	81.38	80.85	81.06	84.29	87.00	87.13	88.00
1919	89.93	90.27	91.17	92.05	91.83	91.55				

In table 4, the median weight of all boys born in 1905 is given for each month of the year without regard to the month of age. The seasonal growth is thereby shown. Thus, in the five months from January to June, 1914, the gain was less than ½ pound, but in the five months from June to November, 1914, the gain was 3½ pounds.

TABLE 5

The monthly increase in weight of boys born in 1905

YEAR	JANUARY TO FEBRUARY	FEBRUARY TO MARCH	MARCH TO APRIL	APRIL TO MAY	MAY TO JUNE	JUNE TO SEPTEMBER	SEPTEMBER TO OCTOBER	OCTOBER TO NOVEMBER	NOVEMBER TO DECEMBER	DECEMBER TO JANUARY
1911	−0.77	+0.70	−0.10	−0.35	−0.02	+3.74		+0.17	+0.10	+1.51
1912	−0.12	−0.08	+0.41	−0.04	+0.04	+1.95	+0.24	+0.76	+0.40	+0.56
1913	+0.55	+0.24	+0.45	−0.13	−0.08	+1.34	+0.85	+0.84	+0.37	+1.08
1914	+0.28	+0.46	+0.19	−0.39	−0.17	+1.42	+1.04	+0.67	+0.74	+0.71
1915	+0.58	+0.30	+0.10	+0.02	+0.22	+2.22	+0.08	+0.64	+0.81	+0.62
1916	+0.79	+0.46	+0.24	−0.26	+0.21			+0.68	+0.71	+0.50
1917	+0.42	+0.40	+0.22	+0.38	−0.02	+1.70	+0.83	+0.95	+0.96	+0.94
1918	−0.29	+1.25	+0.25	−0.53	+0.21	+3.23	+2.71	+0.13	+0.87	+1.93
Average...	+0.18	+0.47	+0.22	−0.16	+0.05	+2.23	+0.96	+0.61	+0.63	+0.98

For convenience we may divide the total of 2.23 pounds from June to September into 0.74 pound for each of the three months.

The errors caused by neglecting the seasonal growth are strikingly shown by comparing a series of weights chosen from table 1 and table 4. Let us take from table 1, twelve weights, beginning with 56.76 pounds, and from table 4, twelve weights, beginning with 56.73 pounds.

FROM TABLE 1—BOYS' WEIGHTS DISTRIBUTED BY MONTHS OF AGE	FROM TABLE 4—BOYS' WEIGHTS DISTRIBUTED BY MONTHS OF YEAR
pounds	*pounds*
56.76	56.73
57.23	57.19
57.74	57.38
58.34	56.99
58.64	56.82
59.51	*
60.01	*
60.54	58.34
60.94	59.38
61.58	60.05
61.56	60.79
61.83	61.50

* July and August; the vacation months.

In these two series, the beginning and the end weights are almost the same, but in the middle of the twelve months the divergence is 2 pounds or more.

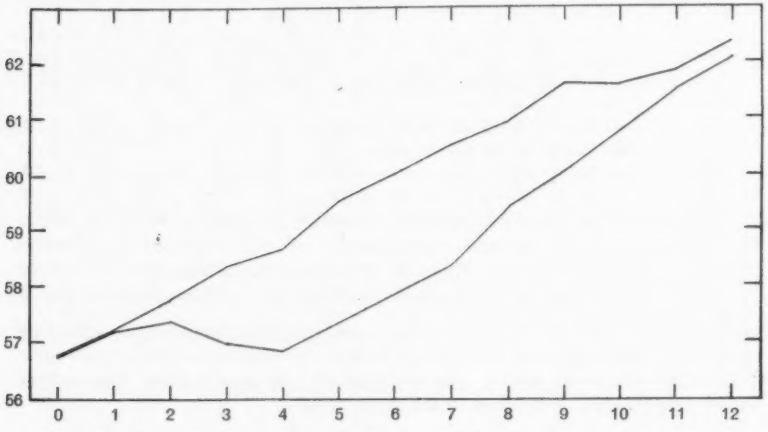

Fig. 3. The ordinates are pounds and the abscissae are months. The lower curve records boys' weights distributed by months of the year (table 4); the upper curve records boys' weights distributed by months of age (table 1).

In figure 3, the two series are shown graphically.

The enquirer will wish to know how the marked seasonal variation in weight is so completely masked in table 1 and figure 1, and in the upper curve of figure 3, examples of the statistical method hitherto in use. The answer is that in the method hitherto in use—in which the only criterion is the month of age—the month of age for half the boys will fall in a season of rapid growth, while for the other half the same month of age will fall in a season of slow growth. Compare during the months of age from 106 to 118 the growth in weight of boys born in August, 1905, with that of boys born in February, 1905 (table 6).

TABLE 6

AGE IN MONTHS	SEASON	WEIGHT	AGE IN MONTHS	SEASON	WEIGHT
Boys born in August, 1905					
		pounds			*pounds*
112	December, 1914	59.96	118	June, 1915	61.56
106	June, 1914	55.25	112	December, 1914	59.96
		4.71			1.60
Boys born in February, 1905					
112	June, 1914	55.75	118	December, 1914	61.17
106	December, 1913	54.50	112	June, 1914	55.75
		1.25			5.42

It is clear that if the age in months is alone considered, the period of rapid growth in boys born in February will coincide with the period of slower growth in boys born in August. The two periods will compensate each other if the February and the August boys are treated *en masse*, as in table 1 and figure 1, and in the standards hitherto so largely used to determine the growth of school children.[2]

IV

Rather than accept a dictum so far-reaching, the reader may here suggest that my entire contention rests on a palpable error—a failure to take into account the heavier garments worn in winter. Table 5

[2] My data do not yet show any seasonal variation in the height of school children.

shows a net increase of 0.76 pounds in the first five months of the year (+0.18, 0.47, 0.22, 0.05, −0.16), whereas the increase in the last five months of the year is 3.92 pounds (+0.74, 0.96, 0.61, 0.63, 0.98). But it will be urged that in May, which falls in the first five months, the winter clothing is laid aside; and in October, which falls in the last five months, it is again put on.

A careful examination of the data will show that the seasonal differences in growth cannot be explained by seasonal differences in the weight of garments worn. Consider the following groups, taken from table 5.

INCREASE FROM		INCREASE FROM		INCREASE FROM	
	pounds		*pounds*		*pounds*
January–February	0.18	June–September	2.23	October–November	0.61
February–March	0.47			November–December	0.63
March–April	0.22			December–January	0.98
Average	0.29		0.74		0.74

Obviously, the second group is composed of summer months and the third group of winter months. The first group requires, perhaps, a word of explanation. In Boston the month of April offers the promise but not the reality of spring. The average temperature rises from 40° on April 1 to 51° on April 30. In 1919, the maximum temperature on April 16 was 42° and on April 25 it was 38°. In only three days of April was the maximum temperature above 61°. It is unlikely that materially lighter clothing is worn during April. For our present purpose April, therefore, should be classed with the winter months.

The figures just presented indicate that clothing is, relatively, a negligible factor. Were clothing an important factor, the weights for June to September would show a decrease, as compared with those for January to April, and the weights for October to January would show a considerable increase as compared with those for June to September. No such fluctuations are apparent.

Observe now the figures from March to June (table 5).

Increase in weight

	Pounds
March to April	+0.22
April to May	−0.16
May to June	+0.05

If a change from winter to summer clothing be invoked to explain the low average of growth from January to April, just discussed, it cannot be used again to explain the failure to grow from April to May, and a third time to explain the failure to grow from May to June. It will doubtless be found, taking the average of eight or nine years, that the change from winter to summer clothing is made within a short period and bears a definite relation to the temperature curve.

Finally, too much emphasis must not be laid upon hypothetical differences in summer and winter clothing. Careful enquiry shows that the indoor clothing of Boston public school children does not change during the school year as much as might be supposed. Such changes cannot explain away the periodic growth demonstrated in this investigation. With every reasonable allowance for error, it seems impossible to deny a seasonal variation in the weights of school children.

V

In figure 2 the seasonal curve departs from the present standard curve by fully 2 pounds, a difference almost three times the average total growth for the first five months of that year. Such deviations justify two deductions:

1. To determine the normal growth in weight, the child must be weighed once a month, or oftener. If the child is weighed less often, the seasonal variation will be missed.

2. True curves of growth in weight demand that the monthly weights be distributed according to the months of the year, and not according to the months or years of age, as is the present custom.[3]

[3] A substantial part of the cost of this study has been paid by the Permanent Charity Fund, whose assistance is gratefully acknowledged. I am much indebted also to the Department of Education in Harvard University for the loan of Mr. L. A. Maverick during the summer of 1919.

CERTAIN CHANGES NOTED IN ERGOGRAPHIC RESPONSE AS A RESULT OF TOBACCO-SMOKING

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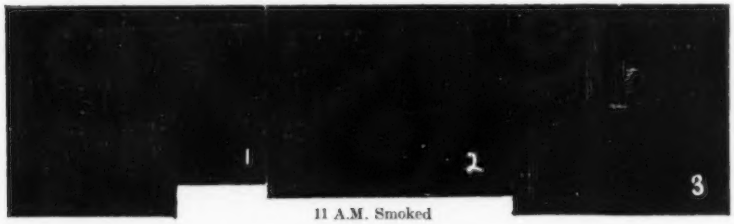
This report is based upon material secured in the course of an investigation undertaken with a view to ascertaining the effect of tobacco-smoking upon voluntary muscular work, as determined through ergographic observation. The study was suggested by Lombard's (1), (2) findings and the procedure followed was essentially that utilized by him (q.v.).

In the summer of 1915 when, for a month, I had the opportunity of association with Lombard in connection with an ergographic study (3) upon which he was engaged at the time, I noted that my own tracings were uniformly free from those periodic recoveries, after the first loss of power, which were consistently characteristic of Lombard's records (1). These recoveries, or returns of power, failed to manifest themselves in my curves in spite of any subjective awareness, or even desire, and continued physical endeavor. The records showed throughout only a gradual falling off of performance registry (fig. 1) from maximal contraction to zero. After smoking, Lombard noted that the initial loss of power came more quickly, to be followed, however, as in his non-smoking records, by the characteristic periods.

In the spring of 1917 I found it possible, personally, to undertake a short study of the effect of smoking upon ergographic response, expecting to encounter, if anything, only a diminution in total amount of work performed. Greatly to my surprise, I noted the consistent appearance of periods practically identical with those characteristic of Lombard's smoking and non-smoking records. Since that time it has been possible to elaborate the results somewhat and, in spite of their apparent slenderness, they were deemed worthy of report.

Prior to the time of undertaking this study, I had been only an occasional smoker, just able to tolerate one or two mild cigarettes with-

out suffering any of the acute tobacco effects. The specific procedure followed in this work was, as indicated, that employed by Lombard (1). The ergographic records were taken every second hour, daily, from about approximately 9 a.m. to 7 p.m., care being taken to look away from the record as it was being taken and, as far as possible, to avoid



11 A.M. Smoked

9 A.M. 1 P.M. Smoked

3 P.M. Smoked

5 P.M. 7 P.M.

FIGS. 1-6

all suggestive influence. After a week of preliminary work to secure adaptation to the schedule and to overcome initial practice effect, the actual smoking records were begun; i.e., a cigarette of medium strength was smoked just prior to the moment of beginning a record, at the expiration of the two-hour interval. Effort was suspended after the first loss of power.

At first, aside from an apparently marked decrease in the extent of the record, no essential change was noted. On the third day of the test, however, it was found that, if effort were continued after the first loss of power, it was possible, in contrast to the results of previous trials, to continue long beyond this point, the curve then showing distinct periodicity (figs. 2, 3 and 4), seemingly identical with that so manifest in Lombard's curves. This finding, it might be well to note, was extremely unexpected and wholly unlooked for. It was found, in addition, that this effect would persist for some hours without additional smoking (fig. 5).

When electrical stimulation was applied directly to the muscles by means of the induction current, essentially as described by Lombard in his Laboratory Manual (4), the weight having been materially diminished, the record showed both with and without the use of tobacco a distinctly unbroken character, marked by no period formation whatsoever, thus indicating the causal locus of the periodicity to be further central than the muscles or the nerve endings. The same findings were noted on nerve trunk stimulation also, thus in both respects corroborating Lombard's personal observations.

Four other subjects, only one of whom had previously smoked to any degree, were obtained. Although these subjects were untrained and the schedule much less thorough, the findings seemed to bear out in a general way those determinable in my own records.

It might be well to indicate, at this point, that these results seem to be at variance with observations made by Hough (5) in tests carried out upon himself, utilizing a somewhat different apparatus.

It appears, therefore, that the essential cause of the periodicity is central nervous system fatigue, or depression, induced in this instance by tobacco products absorbed in the act of smoking. On this basis it might not be impossible to account for Lombard's periodicity, for which, thus far, apparently no satisfactory explanation has been afforded. He reports having already smoked for a number of years when the periods were first noted and has since observed that although, as indicated, the first loss of power comes more quickly on smoking days, the periods always appear sooner or later, and have apparently the same character at all times. In view of the fact that the periods are present constantly, even when smoking had been dropped for a period as long as a month, it is not inconceivable that the long-continued use of tobacco may have, in this case, exercised some more or less permanent change in the substance of the central nervous system, specifically concerned in the mech-

anism of ergographic response. On the other hand, it is equally possible that the periods might have occurred even without smoking, had the central nervous system been originally of a type especially readily susceptible to fatigue. Lombard is unable to state whether any of his eight subjects, two of whom manifested periods, were smokers. In the same way it is conceivable that other central nervous system depressants may produce similar changes in ergographic response.

It is distinctly unfortunate that time and opportunity did not permit more extensive investigation, and with a larger series of subjects, both smokers and non-smokers, and it is primarily in the hope of stimulating further work that this brief note is submitted.

SUMMARY

On the basis of the results obtained in this study, it appears that under the influence of tobacco-smoking, a distinct periodicity may be demonstrated in the curve of ergographic response, arising apparently as a result of central nervous system fatigue or depression.

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DETERMINATION OF THE CAPILLARY BLOOD PRESSURE IN MAN WITH THE MICRO-CAPILLARY TONOMETER

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Physiologists have long appreciated the prime significance of the capillary circulation and in recent times clinicians are coming more and more to realize that this part of the circulatory bed is of importance in connection with purely medical problems. The cardio-vascular system functions for the distribution of the blood but the effective changes pertaining to the nutriment of the tissues occur in the capillary bed. Here the metabolic interchange takes place; food is delivered to and waste is largely withdrawn from the active cells. It is, therefore, important to know the pressure under which the blood is delivered to the capillaries in different conditions of health and disease and to correlate this knowledge with the functional activity in other parts of the vascular bed. At the present time no method is adapted to such a study either in experimental animals or in the human being.

The fundamental observations of Roy and Brown (1) in 1878 on the capillary circulation in the frog were never developed for application to the circulation in the mammal because the method was inadequate to this purpose. Quite recently Cannon (2) has emphasized the significance of the capillary bed in traumatic shock, and Dale and Laidlaw (3) and Dale and Richards (4) in histamine shock. These authors conceive that in shock the capillary walls are injured by circulating poisons so that they lose tone with the result that a large volume of blood is pooled in the capillary area. This pooling of the blood in the capillaries accounts for the primary fall of arterial pressure, which is further accentuated by a transudation of plasma through the injured capillary wall so that there results an actual decrease in circulating blood volume. These two factors are regarded as the basal cause of the circulatory phenomena of shock.

Lombard (5) showed a number of years ago that if a drop of oil be placed upon the skin it is possible to see the underlying capillaries, and

he sought with this information to develop a method of determining the pressure of the blood in these vessels. For this purpose he used a small glass chamber, the floor of which was made of gold beaters' skin with a small hole in the center. The chamber was filled with glycerine and brought into contact with the skin so that it was possible with a microscope to observe through the glass roof and a small opening in the membranous floor the effects which were developed in the underlying capillaries when the pressure on that area of skin was elevated by forcing more glycerine into the chamber. Although Lombard made a number of observations with this instrument, the technique was so difficult that it was not possible of wide application. With Lombard's experience in mind, we have developed a method which we believe is applicable to the study of capillary blood pressure both in animals and in man, and the purpose of this paper is to describe our method and to give the results which we have thus far obtained with its use.

There are some twenty-five articles in the literature bearing upon the determination of the capillary blood pressure obtained by the use of various methods, and the results reported in these articles vary from 7 mm. to 70.5 mm. Hg. for the normal capillary blood pressure in man. It is obvious that if the results by the several methods vary to this extent, they cannot be considered as trustworthy. It is not surprising, therefore, that Friedenthal (6) in a recent review of the subject, reaches the conclusion that capillary blood pressure determinations can be of little practical significance. They are, in fact, of less value than inferential deductions drawn from the values of arterial and venous pressure. Inferential deductions, on the other hand, are certainly not free from objections, as is shown by the following.

Thus Fick (7) thought that pressure in the capillaries is almost as high as in the arteries, and that the greatest fall in pressure occurs in the small veins. His conclusions are based on theoretical considerations.

Following Poisselle it is generally believed that the principal loss of energy occurs in the capillaries because the blood channel is narrowest in the capillary area. On the other hand Campbell (8) has assumed that the greatest fall in pressure occurs in the small arteries and Levy (9) came to the same conclusion as the result of mathematical calculations. Goldmann (10) believes that the principal loss of energy occurs in the arteries. The lumina of the smallest arteries are not much greater than those of the capillaries. The velocity is much greater in the former, however. The result is the great fall of pressure in the small arteries.

Bargolomez (11) has thrown some light on this important question by the following experiments. He cannulated the smallest possible branches of the arteries (middle and posterior auricular arteries) and also the corresponding venous tributaries, and recorded their pressures monometrically. To facilitate the work he dilated the vessels (application of heat) and introduced needle cannulas. Then he allowed the vessels to return to their original caliber before starting his experiment. Thus he found that the normal blood pressure fall in the capillaries is very slight (about 4 mm. Hg. in the rabbit's ear). He concludes that the greatest fall in blood pressure (90 per cent) occurs normally in the arteries of moderate caliber.

It is perhaps as striking to the reader as to ourselves that a trustworthy method of actually determining the pressure in the capillaries is wanting. Let us for a moment review the previous methods employed for this purpose, The majority of them rest upon the principle, introduced by von Kries (12), that the paling of the skin is dependent upon the pressure and amount of blood in the superficial capillaries. von Kries employed a small glass plate of known size and applied it to the surface of the skin and the amount of pressure necessary to cause the skin to pale was interpreted as a criterion of the capillary blood pressure. We shall show that this criterion is inadequate in that the paling of the skin is not necessarily accompanied by a cessation of flow in the capillaries and we believe that it is due rather to an emptying of one or more of the venous plexuses lying in the dermis.

Spalteholtz (13) has published drawings of a reconstruction model of the blood vessels of the skin. These show that there are three venous plexuses in the cutis and one in the subcuticular layer. We believe that as increasing degrees of pressure are applied to the skin, these plexuses are successively more or less emptied of their contained blood with the result that the skin develops varying degrees of pallor. Our observations make it clear that even extreme grades of pallor are not necessarily associated with a cessation of blood flow in the superficial capillaries. It follows, therefore, that pallor of the skin cannot properly be regarded as evidence of capillary collapse.

The principle of skin pallor underlying the method of von Kries was likewise employed by von Basch (14), by von Recklinghausen (15) and by Basler (16). von Basch sealed a small glass capsule on to the skin and determined the pressure necessary to cause paling of the underlying area.

von Recklinghausen's method was essentially the same. It is that which he employed for the determination of the venous blood pressure. Basle[illegible]ed an instrument which he called an ochrometer; this consisted of two closed chambers with glass roofs and floors of gold beaters' skin which was sufficiently translucent to indicate any changes in color in the underlying skin. Two fingers were used, one to observe the degree of pallor and the other for the control. The microscopic fields were brought up to a single eye piece by means of prisms. One of the chambers was then inflated, causing the membranous floor to press upon the skin until the first evidence of pallor, as determined by the control finger, was observed.

von Kries, using his method, gives as the normal capillary pressure 37.7 mm. Hg. This determination was made when the hand was 490 mm. below the crown, a position which we assumed to be approximately at heart level. He likewise reports the capillary blood pressure in the ear as being 20 mm. Hg., and in the mucous membrane of the rabbit's mouth as 33 mm. Hg.

Hough and Ballantyne (17) employed the method of von Kries to study the effect of temperature on the capillary blood pressure. Their readings for the normal ranged from 40 to 50 mm. Hg. at a room temperature of 20–21°C. When the temperature of the surrounding air was reduced to 6°C., the capillary pressure was 65 mm. Hg., and when the temperature was raised to 26°, it was from 50 to 55 mm. Hg. From our own observations we judge that the temperature effects noted by these authors are associated not with actual changes in capillary blood pressure but rather with the behavior of the superficial venous plexus. We believe a significant factor contributing to the pallor of the skin is a decreased amount of blood in this plexus. If the latter is relatively empty and the skin is correspondingly pale, it will require a greater skin pressure to develop a further paling. The paling under these circumstances would then be due to the pressure emptying the deeper-lying plexuses. The resistance to compression of the skin vessels increases as the vessels compressed lie deeper and deeper in the several layers of the skin. It follows, therefore, that when the skin is cold and consequently pale, a greater pressure will be required to produce a further paling than would be the case if the skin were of a normal color. Hough and Ballantyne also report a heightened capillary pressure associated with arterial constriction, hence they thought that capillary pressure was dependent on some other factor than that of the arterial tone. These findings like their findings in connection with the effects of tem-

perature do not agree with our results, and we believe that the latter, as in the case of the former, are associated with the inadequacy of the criterion employed.

Natanson (18) has published two papers using the von Kries method. He took for his standard the complete blanching of the skin and studied, among other things, the effect of mass compression of the arm on the capillary pressure in the hand. Using the criterion of complete blanching of the skin, he found the normal capillary pressure to be 70.5 mm. Hg. The highest capillary pressures were observed when the constricting band exerted a pressure of 42.5 mm. Hg. When the constricting pressure was raised to 52.3 mm. Hg., capillary pressure fell. He assumes that at this pressure both the arteries and veins were being compressed and draws the conclusion that the capillaries cannot be filled with blood except under the influence of arterial pressure. If the latter fails, the capillaries become bloodless and collapse through the effect of a relative rise of tissue tension. We have not been able to confirm the latter observation, and believe that here again the inadequacy of the criterion employed by Natanson is sufficient to explain his results.

Schiller (19) and also Rotermund (20) made use of von Fries's method slightly modified by the addition of Fick's ophthalmotonometer (21). This addition served merely to make the pressure readings easy since they could be read off on a scale calibrated against a spring. Schiller found that the highest capillary pressure (about 40 mm. Hg.) occurred when the temperature of water applied to the skin was approximately that of the skin, namely, 35°C.

Rotermund, using the same method, found the capillary pressure on the skin of the forehead when the subject was in a recumbent position, averaged about 26.8 mm. Hg. He also applied this method to study the influence of age, nutritional state, dyspnea, nephritis and arteriosclerosis on capillary blood pressure.

von Basch applied his glass capsule method to a study of the capillary blood pressure in human beings and upon experimental animals. The criterion was essentially the same as that employed in the von Kries method. He found that the capillary blood pressure in the rabbit's ear did not differ materially from that of human subjects. In the rabbit's ear the pressure ranged from 21 to 25 mm. Hg., and in the healthy human being it was between 25 and 30 mm. Hg. In human beings he observed that a low capillary blood pressure was frequently associated with high arterial tension, but at times the converse of this

was true, namely, that a high capillary pressure was associated with a low arterial tension. His inference from these observations led him to the belief that the capillary pressure is independent of the arterial pressure.

When the chest of an experimental animal was compressed, there occurred a rise in capillary pressure accompanied by a moderate fall in arterial pressure. He interpreted this result as indicating that the rise of capillary pressure followed venous stasis. Similarly in man chest compression produced a moderate rise of capillary pressure with a corresponding fall of arterial pressure. The fact that the capillary pressure rose less in man than in the experimental animal suggested that the venous stasis was less extreme. He also cut the cervical sympathetic nerve in the rabbit and found an increase in capillary pressure associated with the slight fall of arterial pressure. This pointed to a dilatation of the arterioles of the rabbit's ear and was confirmed by direct inspection. The injection of strychnine caused a rise of arterial pressure with a fall of capillary pressure; in other words, the converse of the above experiment. This was interpreted to mean that a constriction of the arterioles had occurred.

von Basch formulated the following hypothesis: that the degree of capillary pressure rise may serve to differentiate between venous stasis and arteriolar dilatation. While the former raises capillary pressure markedly without altering arterial blood pressure, a condition of arteriolar dilatation produces a moderate increase of capillary pressure with a corresponding fall in arterial blood pressure. He applied these results clinically and obtained data which he regarded as of distinct value in both diagnosis and treatment.

Using Hooker's (22) capsule with the criterion of paling, Briscoe (23), in a study of the Raynaud phenomena, in cases of "irritable heart," found that when the hand was cyanotic the capillary pressure was 36.9 cm. of water as compared with the normal controls which gave a pressure of 23.5 cm. of water. In individuals subject to vasomotor changes when the color of the hand was normal, the capillary pressure was 25.3 cm. of water, and that when the same hand was blue the capillary pressure was increased to 33.3 cm. of water.

von Recklinghausen gives as the normal capillary pressure in the finger tip with the hand at heart level, a reading of 52.5 mm. Hg.

Basler's ochrometer has been used by Basler himself and by a number of other observers. The normal capillary pressure as given by Basler is about 7 mm. Hg. Goldmann, using this method, concludes

that the normal capillary pressure is about 8.5 mm. Hg. A moderate rise in external temperature causes no appreciable change in the capillary pressure, but if the external temperature be raised 10°C. or more, the capillary pressure is increased.

Landerer (24), using Basler's ochrometer, found that the normal capillary blood pressure ranged from 17 to 25 mm. Hg. During a cold bath the capillary pressure fell while the arterial pressure rose. In a warm bath the capillary pressure was unchanged while the arterial pressure fell. Landerer, Krauss (25), Friedenthal and others have used this method in clinical cases.

Krauss studied the capillary pressure in circulatory disturbances (valvular disease, myocardial disease, venous stasis), pulmonary diseases (emphysema, chronic bronchitis, tuberculosis), status asthenicus, severe anemia, carcinoma and cachexia. Besides the ochrometer he also used an apparatus of his own (a microscopic method) and also Weiss' blood method. He obtained some very interesting results. In brief, they confirmed the older observations of von Basch and Rotermund and agreed with those of Landerer.

The foregoing methods are all based upon the principle of paling of the skin originally introduced by von Kries. A decidedly different method has been employed by Basler and by Weiss. Weiss's (26) method consisted of pricking the skin and observing the pressure necessary to stop the exudation of blood through the wound. The finger tip was enclosed in a chamber containing fluid in which the pressure could be raised. It was necessary, of course, to insure that bleeding continue after the pressure was removed.

Basler (27) pricked the volar surface of the middle finger with a needle; he then sealed a piece of rubber tubing onto the finger (by applying a hot rod against the edge of the rubber). This formed a little chamber in the center of which was a bleeding spot. The chamber was filled with hirudinized saline solution, and closed with a roof. Now the pressure of the exuded blood was registered manometrically. His recorder was of the lever type and rather delicate. He named this apparatus the "Hautmanometer." The determinations made with this apparatus agreed quite closely with those of the ochrometer.

Both of these blood methods are faulty because it is not possible to know the depth of the wound, since the skin thickness is so variable and consequently the size or the depth of the vessels punctured is a matter of chance.

The theoretical objections to the various methods above discussed together with the fact emphasized by Friedenthal that the readings obtained by these methods vary by more than 100 per cent indicate clearly that they have little or no practical application. In order to be sure that the readings accurately represent the pressure in the capillaries themselves, a criterion similar to that employed by Roy and Brown, namely, the visual determination of the point at which the capillary flow ceases, is essential. Using a tonometer similar to that which we have employed and the criterion of cessation of corpuscular flow, these authors obtained pressure readings in the capillaries and venules of the web of the frog's foot of 7.3 to 11 mm. Hg.

Lapinski (28) employed the Roy-Brown method to study the effect of nerve section on the capillary pressure in the frog. He obtained no very clear-cut results; however, his determinations of normal capillary blood pressure in small frogs was between 15 and 44 mm. Hg. and in large frogs between 30 and 60 mm. Hg.

Natanson, in his studies on the effect of mass ligature, controlled his observations on man by experiments on the frog. He found the normal capillary pressure varied from 12 to 24 mm. Hg. in the different capillaries of the frog's web.

That such a criterion may be applied in the case of the human subject is indicated by the observation of Weiss (29) who, using oil on the skin as suggested by Lombard, first observed corpuscular flow in the human capillaries. We have developed an instrument applicable to man and experimental animals which permits of the accurate use of this criterion.

If a drop of oil be placed on the skin of the hand and the area so treated be examined with the microscope under a strong light, as, for example, a 40 Watt electric bulb placed close to the microscope objective, one may readily see the capillary tufts scattered through the field. Most of these tufts come up from the deeper parts of the dermis, fold over and return so that only a small part of the capillary loop can be brought into focus. Under such conditions it is scarcely ever possible to see the corpuscular flow. Occasionally a capillary may be found which, arising from the depths of the dermis, turns at right angles and takes an horizontal course for a short distance, and in such a case, under favorable conditions, the corpuscular flow may be observed. Such capillaries are so rare, however, on the general surface of the skin, that they do not serve for pressure determinations. In the skin overlying the matrix of the finger nail however, many capillaries take such an

horizontal course and in a single microscopic field a number of these vessels may be brought into focus at one time. Here one frequently sees corpuscular flow without offering any resistance to the blood stream. If a constricting pressure be applied to the upper arm the corpuscular stream may be seen in many of the capillaries without difficulty. A drawing of the picture seen is given in figure 1. The sharply outlined capillaries are seen as red threads lying on a pink ground. The picture is very much clarified if the skin be first scrubbed with soap and water and afterwards thoroughly dried. This procedure removes the loose epidermis and softens the tissues.

The methods based on this principle introduced by Lombard are the Krauss method, Basler's kapillar-tonometer (30) and Kylin's method (31).

In Krauss's method the capillaries are visualized through a simple lens (magnifying 10×). Krauss takes as his criterion the disappearance of the capillaries. We have employed such a method but have not succeeded in making the capillaries disappear at pressures corresponding to those of Krauss. We believe that the paling of the skin (which occurs at very low pressures) and the minute size of the capillaries as seen at such a low magnification may give the impression of a disappearance of the capillaries when even slight pressure is applied. When controlling this method with our own (in which a magnification of at least 68 is used) we have been able to see distinctly capillaries which were hazy and almost invisible with a magnification of ten.

Concerning Basler's tonometer we may say that except for the fact that the gold beaters' skin has no circular opening in its center, it is almost identical with that of Lombard. The glycerine reservoir and the fact that the chamber is filled with glycerine, is common to both methods. An added difficulty in the method of Basler may be the collection of air bubbles within the chamber which will disturb the clarity of vision. In our experiments in the early part of 1919 we used chambers which were almost the counterpart of Basler's, but have found them difficult to work with and for reasons of practicability have discarded them. Basler's criterion is the disappearance of the capillaries. We believe this to be incorrect in principle and difficult of execution in the majority of instances. In the publication dealing with this method he gives no results obtained by its use.

Kylin's method, which was presented at the Ninth Nordiske Congress of Internal Medicine held at Copenhagen, August, 1919, depends on the visualization of the capillaries. Unfortunately we have been unable to

obtain any exact information about his method, his criterion or his results.

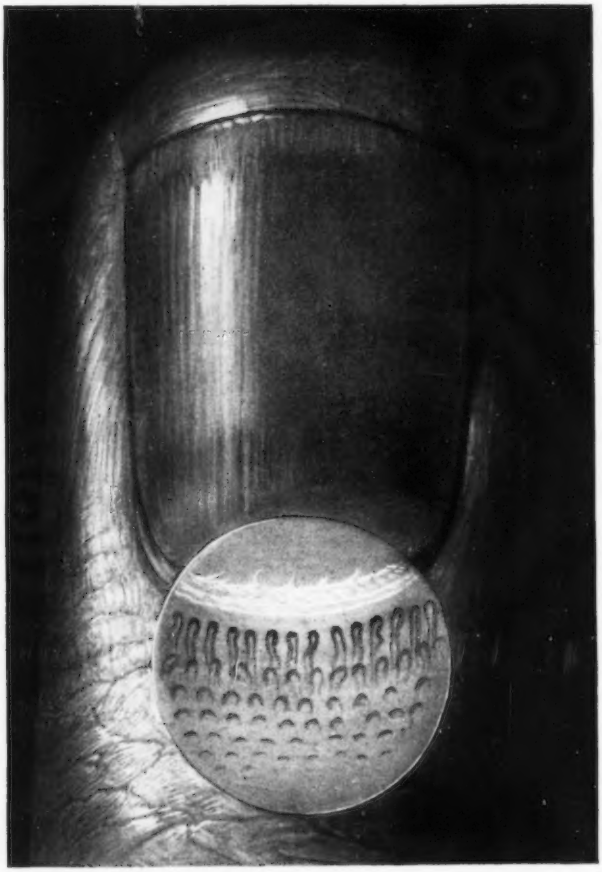

Fig. 1. A drawing of the finger tip to show the area of skin at the base of the finger nail which is used in the determination of the capillary blood pressure. The circle overlying this area represents the miscroscopic field when the skin is observed under a drop of oil with the aid of a strong light. The capillaries are slightly diagrammatic: a single focal plane will not bring them all into focus at one time.

The micro-capillary tonometer. The instrument which we have used is shown in figure 2. It consists essentially of two adjusting devices. Screw *1* permits of raising and lowering the finger rest so that the area of skin under observation may be brought into an horizontal plane. Screw *2* permits of adjusting the pressure capsule in suitable contact with the skin. The finger rests on plate *3*, which rocks so that the finger tip may assume a comfortable position. The instrument is placed on a microscope stage and when the forearm is supported with a comfortable rest, the subject is sufficiently comfortable, so that there

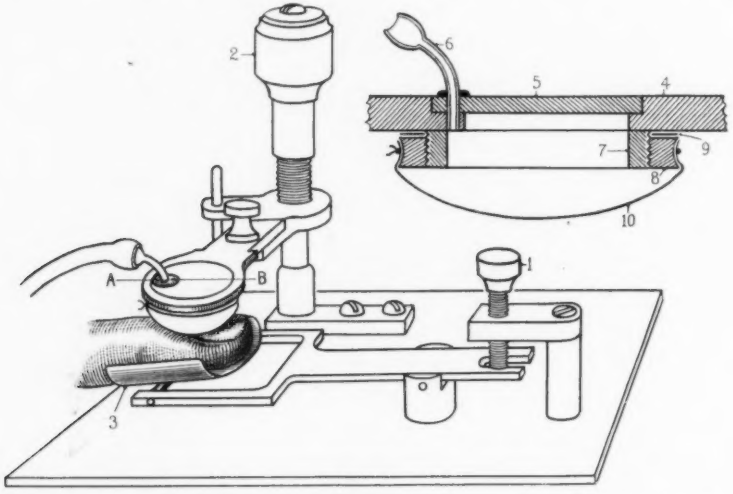

Fig. 2. The micro-capillary tonometer The instrument is of such a size that it rests on the microscope stage. Description in text.

is no movement or tremor of the finger. The pressure capsule is shown in the insert figure, which represents a cross section along the line *AB*. It consists of a brass ring, *4*, into the upper surface of which is sealed a thin glass plate, *5*. Through the latter passes tube *6*, which serves for the inflow and egress of air. To the lower surface of plate *4* is attached a brass ring, *7*, threaded on the outside to receive the collar, *8*. The latter screws air-tight against the washer, *9*. Over the brass collar *8* is tied the gold beaters' skin, *10*. The metal tube, *6*, is connected through a rubber tube with a rubber ball of about 200 cc. capacity, which may be compressed between two plates by means of

an adjusted screw, a device similar to that which is ordinarily used for calibrating the Hürthle manometer. A bypass on the tube connecting the capsule with the rubber bulb leads to a single arm mercury manometer, by means of which the pressure in the chamber may be determined.

After the finger has been brought to a comfortable position on the finger-rest with the area for study in the horizontal plane, the pressure capsule is lowered until the gold beaters' skin comes softly in contact with the upper surface of the finger. The gold beaters' skin is tied on sufficiently loosely so that when the air is forced into the capsule it exerts pressure upon the skin without loss of pressure due to the tension on the membrane.

Preparation of membrane. The successful use of this device depends upon obtaining gold beaters' skin which is free of porous openings, is entirely soft and pliable and is transparent. We have found that gold beaters' skin prepared according to the following directions fulfills these requirements. The membrane is first washed in tepid water to remove all powder and dust which may be adherent to the surface. This cleansing is facilitated by rubbing both surfaces of the membrane with the ball of the finger or with a piece of absorbent cotton. It is then rinsed several times in clean water, after which it is ready for use. The brass collar, *8*, in figure 2 is now removed from the instrument and screwed down over an obturator which, presenting through the collar, has an oval contour such that when the membrane is tied into position it will lie somewhat as shown in the detail. With the collar in position on the obturator the membrane is laid over the top and tied securely with several turns of silk thread. The collar is now returned to the instrument, when it may be tested to see whether or not the attached membrane is perfect. If it stands a pressure of 50 mm. Hg. without leak, it is suitable for further treatment.

The collar with the attached membrane is now drained free of water and placed in pure glycerine for 24 hours, after which it is removed, drained free of glycerine and placed in castor oil. The preparation should be left in castor oil for at least 24 hours and may be left in the oil indefinitely. It is thus possible to prepare a number of membranes at one time and to keep them in castor oil until such time as they may be required.

When a membrane is required for use, the ring to which it is attached is removed from the oil and screwed into position on the instrument, a test being again made to be sure that the chamber is air-tight. If a

leakage occurs at this point it is probably due to an imperfection in the washer.

If now the adjustment to the finger be made and the area of skin beneath the member be observed with the microscope, it will be seen that the membrane is entirely transparent so that there is no difficulty in observing the underlying capillaries. Failure to see the capillary field plainly indicates that the membrane is clouded, in which case it should be discarded. This condition is not likely to be found when using a new membrane. A membrane, however, which has been used repeatedly and exposed to air and dirt is liable in the course of time (weeks) to deteriorate and if there is any difficulty in obtaining clear vision of the field it is probable that the transparency of the membrane is at fault. When the instrument is not in constant use it is advisable to return the membrane to castor oil. With reasonable care a membrane should last indefinitely.

A second technical requirement is that the skin should be clean and free from moisture. It should be scrubbed lightly with soap and water and thoroughly dried. The oil on the skin which makes the underlying capillaries visible probably serves by its intimate penetration of the epidermal layer to do away with light reflection from the uneven surface. If then moisture intervenes at the oil-skin boundary, the field is less distinct. It sometimes happens in prolonged observations that the clarity of the field is lost. This may be due to the excretion of sweat. If the oil be wiped off and the finger be thoroughly dried a fresh drop of oil will make the field as clear as before.

In using the instrument we have found it desirable to employ a microscope giving a magnification of approximately 70×. The essential point is to magnify enough to readily visualize the movement of the red cells when the stream is slowed without undue loss of definition and depth of focus. Leitz objective 3 and ocular 1 fulfill these requirements. To use such a lens combination with a rather short working distance, the skin with the overlying oiled membrane must be brought quite close to the glass roof of the chamber. Care must be exercised, therefore, that in manipulation the membrane shall not touch the glass. If this occurs one must dismount the collar and clean the glass, otherwise the capillary picture will be clouded. It is of distinct practical help to one using the instrument for the first time, to first become familiar with the location and appearance of the capillary bed under the microscope. When this is done it is a simple matter to locate the proper field with the chamber in position.

We have experimented with chambers of different diameters but have found that this is a matter of no consequence in obtaining correct readings and we now use for routine observations a chamber of such a size that the brass collar is 25 mm. in diameter. In order to insure that the pressure within the chamber is all transmitted to the underlying skin, it is advisable to make several determinations of the capillary pressure with the chamber at slightly different vertical positions. The position of the chamber which gives the lowest capillary pressure reading is the correct one. With a little practice one makes this adjustment readily and there is no necessity for such preliminary observations. The purpose is, of course, to insure that none of the pressure is lost in the resistance offered by the membrane itself.

Corpuscular flow. With the chamber in position over the finger, when there is no pressure exerted on the underlying skin, the picture is frequently disturbed by reflections of light from the folds of the membrane. This disturbing feature is at once removed if a pressure of 2 or 3 mm. be applied, a procedure which has the effect of smoothing out the membrane over the surface under observation. When the area is first observed it is uncommon to obtain any indication of corpuscular flow. If now the pressure within the chamber be slowly raised by compression of the rubber bulb, the corpuscular flow becomes evident and the red cells are seen streaming slowly through the capillary. As the pressure is further raised this corpuscular flow becomes slower and slower until there is no further continuous forward movement of the corpuscles. At this time one frequently sees a to-and-fro movement of the corpuscles without progress. At first thought this phenomenon would seem to be associated with a transmitted pulsation from the underlying vessels. It may however be due to rhythmic contractility of the capillary endothelium. As the pressure is further raised, this to-and-fro movement of the corpuscles ceases. Now a further increase in the pressure acting on the capillaries may cause the corpuscles to travel in a reversed direction, that is to say, from the venous side toward the arterial side. We are able to offer no satisfactory explanation of this fact at the present time. If now the pressure be slowly lowered the reversed flow ceases and the corpuscles again stand still. Then the to-and-fro movement of the corpuscles is seen, and finally with a further lowering of the pressure the corpuscles stream forward in the normal direction. The pressure at this instant is taken to represent the capillary blood pressure and our reading is therefore made at this point.

The pressures at which the above events occur in one and the same capillary are depicted in figure 3 and again for a second subject in figure 4. The plotted lines in each figure represent several observations on a single capillary. Note that the skin pales in both subjects at a pressure of about 8 mm. Hg., while the corpuscular stream is not slowed until the pressure has been raised to 18 mm. Hg. in one case (fig. 3) and 27

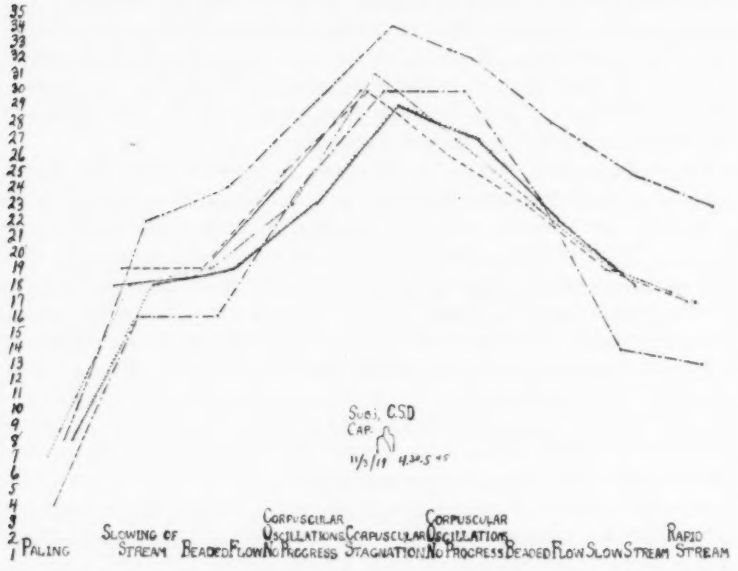

Fig. 3. To illustrate the pressures at which the several events in capillary blood flow occur. The figures along the abscissa represent pressure in millimeters of mercury. The ordinates divide the chart according to the observed behavior of the corpuscles with an increasing and then with a decreasing pressure. All the observations were made on one capillary, the several plotted lines representing single observations.

mm. Hg. in the other (fig. 4). The slowing of the corpuscular stream undoubtedly represents the condition when the pressure without approximates the pressure within the capillary. It is clear, therefore, that paling of the skin is a wholly inadequate criterion of capillary blood pressure. These figures indicate further that repeated observations of the same capillary agree closely in the pressures at which the events under discussion occur. Experience has taught us, however, that the

slowing of the corpuscular stream which occurs with a rising pressure is much less readily appreciated than is the quickening of the stream which occurs with a falling pressure. Consequently we have found it expedient to raise the pressure until the forward movement of the corpuscles ceases and then to lower it, giving close attention to the behavior of the corpuscles. As the pressure falls the corpuscles at first progress slowly and with a further slight lowering of the pressure there is a sudden sharp acceleration which serves as a sharp criterion and

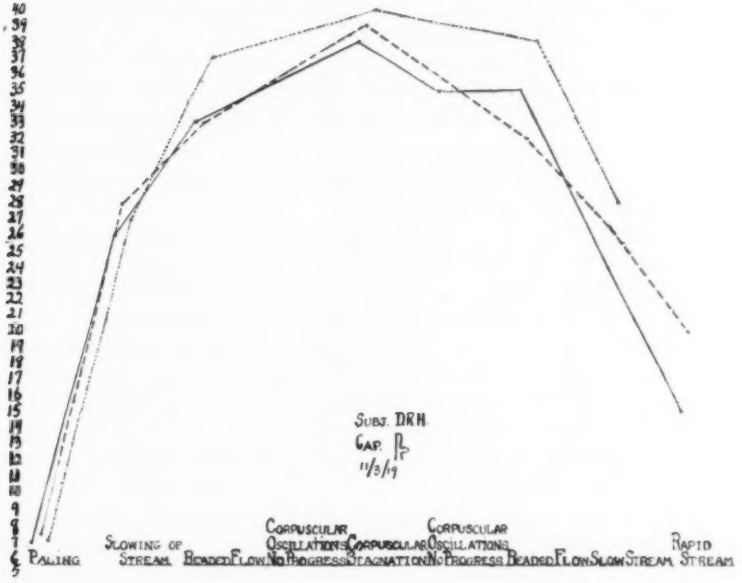

Fig. 4. See figure 3.

one which is easily recognized. The pressure then represents what we have taken to be the capillary blood pressure.

During the procedure above outlined, the capillaries are sometimes emptied of red blood corpuscles, but more often this is not the case, that is to say, when the pressure applied to the skin is sufficient to stop completely the corpuscular flow, the cells are still present in the capillary loop. This observation goes to show that the assumption made by von Kries and other earlier observers that the paling of the

skin is associated with an emptying of the lumina of the capillaries is incorrect. Furthermore, one may readily appreciate that the paling of the skin associated with the application of pressure becomes very extreme even before the corpuscular flow is distinctly slowed. Observations of this kind have led us to the conviction that the criteria used by most other observers are wholly inadequate to represent true capillary blood pressure.

It frequently happens that when one obtains a focus of the capillary field, one or more of the capillaries will stand out conspicuous and large. Such capillaries represent vessels in which the corpuscular flow is stagnated. The individual corpuscles in such a capillary cannot be made out; apparently they are thickly packed together. The pressure may be raised very considerably without changing in any way the appearance of such a vessel. This fact accords with the original observations of Roy and Brown which have been abundantly confirmed by later observers, that all the capillaries in a given vascular bed are not necessarily functioning at the same time.

The idea that not all of the capillaries are functioning at all times is indeed not a new one. Worm-Müller (32) in 1873 in studying the influence of blood volume on the arterial pressure comes to the following conclusion: "Under normal conditions (most likely in every part of the body) a large number of empty or poorly filled capillaries stand ready to respond to the needs of the blood stream." He also spoke of the dilatation of the capillaries resulting from the transfusion of blood. He found on post-mortem examination that the arteries and veins were not overfilled after transfusion. He therefore assumed that the blood collected in the capillaries.

Heubner (33) in 1907 saw many new capillaries open up as the result of the injection of gold sodium chloride into frogs, rabbits, cats and dogs. The experiments on the frogs are described in greatest detail. In these he looked at the frog's mesentery microscopically and injected an amount equivalent to 0.25 mgm. of metallic gold and in one-half to one minute saw the animal practically bleeding into its capillaries. He estimates that the number of capillaries visible after the injection is three to four times as many as before.

Concerning Dale and Laidlaw's work little can be added to what is so generally known about their researches on "Histamine Shock." By a series of ingenious experiments these workers have attempted to prove *a*, the active functioning of the capillaries; *b*, the independence of the capillaries from the rest of the vascular system; *c*, the capillo-

dilating effect of histamine. They were led to these problems by the discrepancy between the effect of histamine in the intact animal and on the excised arterial or uterine strip. In the former it lowered the blood pressure and in the latter it increased the tone of involuntary muscle strips. Dale and his co-workers explain the marked fall in arterial blood pressure which occurs in the intact animal and which cannot be due to a relaxation of arterial tone since histamine causes the isolated artery to contract, as due to a specific dilator action of histamine on the capillaries. Accordingly the capillary beds throughout the body are flooded and the circulating blood is insufficient to fill these areas and at the same time sustain the arterial pressure in spite of a concomitant increase of arterial tone. Whereas the experiments are very suggestive the crucial experiment, namely the effect of histamine on the capillaries as observed directly (through the microscope) is as yet lacking.

Krogh (34) has thrown considerable light on this subject by counting the number of capillaries in a definite area of muscle tissue in the resting state and immediately after exercise. He examined both fresh and fixed tissues. He found that the working muscle showed many more capillaries than the resting tissue. Hence, the conclusion that many new capillaries open up when necessary (e.g., in the working state).

It is obvious perhaps that we avoid the term capillary collapse. We have done this because of a good deal of experimental evidence (particularly that of Roy and Brown) which indicates that capillaries do not collapse when compressed. Why then do we not take capillary emptying (i.e., when corpuscles travel back from capillaries into the arterioles) as the criterion? The reason is that in a number of capillaries it is necessary to raise the pressure acting upon them much above that which will cause the corpuscles to stagnate. Hence the readings would be much too high. This is due to the fact that blood is piling up in the patent capillaries as each neighboring one is emptied of its contents (von Recklinghausen). We believe also that as the externally applied pressure rises the arterioles and capillaries are being simultaneously compressed. On this account pressure determination in a great many capillaries may be impossible with the methods of Lombard, Krauss or Basler. Krauss himself admits this difficulty. The impression that we have received from Doctor Lombard personally concerning some of the drawbacks of his method are to the same effect.

TABLE 1

Observations of the capillary blood pressure in normal individuals

SUBJECT	AGE	HEIGHT	WEIGHT	+ OR − IDEAL WEIGHT	READINGS	CALCULATED HYDROSTATIC DIFFERENCE	CAPILLARY PRESSURE (AVERAGE)	REMARKS
			pounds	*pounds*	*mm.* Hg.	*mm.* Hg.	*mm.* Hg.	
1	20				20, 22		21.0	
2	21	5′ 8″	150	− 4	20, 20, 18	−1.2	19.0	
3	21				23, 23, 19, 18, 22		21.0	
4	21	5′ 8″	135	−19	22, 26, 25, 26, 24, 26, 28, 26	−1.6	25.0	
5	22	5′ 11″	135	−25	27, 27, 28, 24		26.5	
6	22	5′ 6″	150	+ 7	23, 25	−2.2	24.0	
7	21	5′ 10½″	150	−18	17, 18, 31, 16, 18		18.0	
8	21	5′ 10″	158	− 7	20, 22, 19, 23, 18, 24, 25, 25, 25	−1.4	22.3	15, 17 High Art. Pr. 150–180
9	22				17, 22.5, 23.5		21.0	
10	22				25, 23.5, 23.5, 22.5		23.5	16, 15
11	22				25		25.0	
12	24				27, 26, 24, 23, 23		24.5	
13	25	5′ 8½″	155	− 2	22, 18	−2.2	20.0	
14	26	5′ 3″	110	−17	19, 22, 21, 21, 24, 22		21.5	14, 15
15	26	5′ 4½″	140	+ 5	22, 23, 19, 21, 23, 24, 24, 23, 22, 22, 22, 23, 25, 26, 27	0	23.0	
16	31	5′ 10½″	158	−10	24, 27, 27.5, 28		26.5	
17	31	5′ 10½″	150		22, 24, 19, 21, 19		21.0	
18	33	5′ 1½″	135	+17	19, 23, 24	0	22.0	
19	34				24, 23, 22, 21		22.5	

20	47	5′ 8″	140	−14	20, 21, 23, 22, 18, 22		21.0	
21	42	5′ 7″	135	−13	24, 20, 23, 23, 22, 24, 25, 23, 24, 26 24, 22, 22, 22, 24, 23, 26, 23, 22, 23		23.	
A	22	5′ 4″	155	+23	35, 30, 30	−1	32.0	Membrane was cloudy that day and these subjects were difficult to work with
B	30	5′ 10″	157	− 8	27, 32, 27	−1.6	29.0	
C	21	6′ 0″	195	+19	12, 13	−4.8(?)	12.5	Subject difficult to work with; cap. seem to disappear from view very readily. Subject has cold
D					15, 15, 14, 15.5, 15, 15, 15, 13	−2.5	15.0	Subject has a cold. These readings taken in early part of these studies. Technique?
I	16½				22, 16, 16, 18, 18		Corrected 18.0	
II	11				14, 16, 15, 11.5, 11	+4	17.5	
III	11				18, 16, 15	+4	20.0	
IV	10				19, 20, 17	+6	23.0	
V	8				15, 18, 20	+8	26.0	Subject to paroxysms of acrocyanosis
VI	8				16, 19, 16	+8	25.0	

Because some capillaries exhibit no corpuscular flow and because there is an appreciable variation in the pressure in several capillaries in a given area, we have found it expedient to make pressure determinations in five or more capillaries and to average the results. This will minimize the possibility of striking capillaries of extreme pressures. The majority of our determinations, therefore, represent such an average.

Capillary blood pressure in normal individuals. Twenty-five adults and six children were studied. All of the determinations were made with the subjects in the sitting posture, with the hand a little below the heart level. The average capillary pressure in our series was 22.2 mm. Hg. The ages of our subjects ranged from 8 to 47 years. Of the subjects studied the lowest average capillary pressure was 17.5 mm. Hg., the highest was 26.5 mm. Hg. These results are given in table 1.

Four of our cases (A, B, C, and D in table 1) cannot be grouped with the remaining ones, because of the existence of infections (colds) in two of them and on account of technical difficulties in the other two which occurred at the inception of the work.

In some of the cases the distance betwen the level of the hand and the heart level was determined. Like von Kries, von Recklinghausen and Goldmann, we found that capillary pressure varies with the distance between the hand and the heart level. The difference in the pressure readings taken when the hand is at heart level and when the hand is above or below the heart level does not, however, correspond to the hydrostatic differrence in these positions. This is clearly brought out by the following table given by von Kries (35).

HAND CHANGED FROM HEART LEVEL TO	DIFFERENCE IN CAPILLARY PRESSURE	CAP. PRESS. DIFFERENCE / HYDROSTATIC DIFFERENCE
	mm. Hg.	*per cent*
205 mm. below heart level	65	33
285 mm. below heart level	116	40
350 mm. below heart level	225	64

The hydrostatic factor is thus a disturbing element in the determination of capillary blood pressure at the present time. It is therefore advisable that determinations of this pressure should be made at heart level although the inconvenience of such a procedure is considerable. Our readings unfortunately do not conform to this specification, nevertheless the range between the determinations on different subjects is remarkably small. In the case of children our readings run appreciably

lower than in adults but when correction is made for the hydrostatic factor the results correspond closely with those obtained in older individuals. Similarly the results in the group of adults show no relationship between age and capillary pressure. We have not noted in the table whether the subjects were men or women because here also we found the sex factor to be of no significance. On the whole, therefore, the data which we have thus far collected point to a very remarkable constancy in the capillary pressure in normal individuals of both sexes regardless of their ages. Krauss also found that the capillary pressure in children was practically the same as in adults.

Concerning the readings obtained in the individual capillaries, it may be seen that they run within fairly close limits. There are, however, capillaries whose pressure determinations are definitely outside of these limits. We do not average these extreme ones with the rest. The capillaries of higher pressure may be the ones lying deeper down in the skin or those of larger bore. Possibly they represent the piled-up blood in the patent capillaries resulting from the emptying of the neighboring capillaries, as suggested by von Recklinghausen. We discard very low readings when they occur in but one or two capillaries. Theoretically these are the most accurate. For practical reasons, however, we have arbitrarily eliminated these infrequent (low pressure) capillaries and have adopted instead another criterion which is quite as accurate as the former, but more easily carried out. That series of six or more capillaries of the lowest pressure is taken, in which the difference between the extreme capillaries of the series is not more than 6 or 7 (less is preferable). We warn against averaging capillaries of very low or very high pressures with the series. Let us take a concrete example.

Capillary pressure in separate capillaries....

12	eliminate—too low
13	
19	
21	
25	
20	
23	
23	
32	eliminate—too high
20	
38	eliminate—too high
41	

Thus the series consists of { 19, 21, 25, 20, 23, 23, 20 }

$\frac{151}{7} = 21\frac{4}{7}$ mm. Hg. = average

Our figures for normal capillary blood pressure (with the subject in sitting posture, hand below heart level) accord with those of Lombard (18 to 22 mm. Hg.), Landerer and Krauss. The latter two investigators (using Basler's ochrometer) obtained readings varying from 17 to 25 mm. Hg. on the normal individuals.

In our table we have also put down the difference between the actual and the ideal weight of the subject with the idea of noting the effect of over- or under-nutrition on the capillary pressure. Thus far we have nothing definite to say on this subject.

The effect of temperature on capillary pressure. Cold. Cold towels with pieces of ice placed between the layers were wrapped around the arm. The cold was not directly applied over the capillary area, hence the results are not to be interpreted as the direct effect of cold on the capillaries themselves but rather as a reflex effect. The duration of the application was 15 minutes. The results are graphically presented in figure 5. Two neighboring capillaries represented by the continuous and the broken lines were studied. Immediately on the application of cold there was a definite drop in the capillary blood pressure. When the cold was removed a very prompt rise of capillary blood pressure, which was maintained for about 13 minutes, occurred. Then there was a marked drop to a point slightly above normal, then another rise and finally a fall. The occurrences after the removal of cold represent the reaction after such a procedure.

It is striking how closely the curves of the individual capillaries follow each other. Only at one point do they diverge. The explanation for this would seem to be a change in the lumen of one of the capillaries independent of that of the arteriole since both capillaries were in all probability supplied by the same arteriole.

Heat. Heat was studied by means of an electric pad which was wrapped around the arm and kept on for 26 minutes. We made observations on six neighboring capillaries. The electric pad was set so as to create moderate heat. The effect produced by the application

of heat is also shown in figure 5. Almost immediately there was a rise in capillary blood pressure which was maintained throughout the period during which heat was applied. Upon the removal of the pad the pressure in the capillaries fell to a point below the normal value. Soon the pressure average in the six capillaries observed was as before the experiment. One is amazed at the striking parallelism of the pressure curves of the whole group of capillaries. Only here and there does a disparity become evident. For example, in the last phase two capillaries show a fall in pressure while two others show a simultaneous rise in pressure,

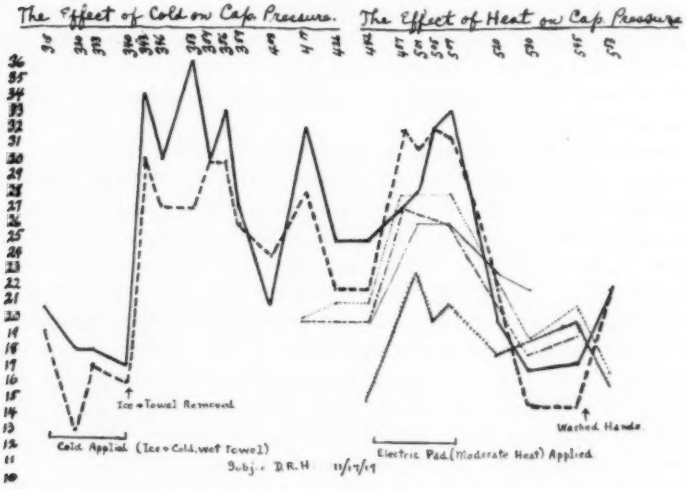

Fig. 5. To show the effect of cooling and warming the fore-arm on the capillary blood pressure in the finger. Two capillaries were followed in the application of cold and six in the application of heat.

although the average in the whole series is practically normal. Here again we must assume a contraction or dilatation of the capillaries themselves. There were occasions when we felt that the diameter of the capillaries underwent changes in the course of our observations, yet most of our evidence supporting the idea of capillary contractility is indirect and sometimes necessarily assumed. In the heat experiment the electric pad was not placed directly over the capillaries but over the forearm and hand. Here again the results are due, therefore, to a reflex effect.

In these experiments the capillary pressure responded to the application of heat and cold as if the stimulus produced vasodilatation and vasoconstriction of the feeding arterioles. We assume this to be a reflex effect from the spinal centers. Krogh (36), however, is reported to have shown that contraction of the capillaries may be evoked by reflex stimulation after the sensory nerves have been cocainized, an observation which indicates an axon reflex. In our experiments, however, the stimulus was applied at some distance from the field of observation so that it seems improbable that the results are due to axon reflexes. The suddenness with which the capillary pressure changes, particularly after removal of the stimulus, excludes the hypothesis that the results might be due to differences in the temperature of the blood. It would be possible to differentiate between a true reflex and an axon reflex effect if the observations were repeated on a subject with impaired cutaneous sensibility.

The figure (fig. 5) shows a remarkable over-compensation of the capillary pressure after the stimulus is removed. Within three minutes after the cold was removed the pressure rose from 17 to 32 mm. Hg. and remained at this high level for a period of 15 minutes, after which it began to fall. Thirty minutes later it was still above the original value. A similar though less striking effect is seen to follow the application of heat. These changes in capillary pressure undoubtedly occur when the temperature of the surface of the whole body is altered. We have no notes indicating a definite change in the caliber of the capillaries under such conditions although, of course, when the capillary pressure was low the corpuscular stream was frequently seen without the application of external pressure.

Our observations correspond pretty well with those of Goldmann. The relative crudeness of his method, however, prevented him from investigating it in such detail as has been done here. His readings (taken with Basler's ochrometer) were definitely lower than ours.

Hough and Ballantyne, who studied the effect of temperature on capillary pressure by means of the von Kries method, found that when the temperature of the external air was reduced from 20°C. to 6°C. the capillary pressure rose from 40 or 50 mm. Hg. to 65 mm. Hg. This was probably due to the constriction of the more superficial vessels and to the fact that the first noticeable color change in the skin was produced when the deeper vessels were compressed. This again illustrates the danger of working with a method depending on skin pallor. This error is clearly the result of the fallacy of the method.

When the temperature of the external air was raised to 26°C. the capillary pressure was 50 or 55. In other words, very little (if any) change occurred. Here again the inadequacy of the method becomes evident.

Schiller also studied the effect of temperature on capillary pressure by means of the modified von Kries method (with Fick's ophthalmotonometer) but his results are difficult to interpret because of the reasons given above. Briefly they are as follows: the highest capillary pressure (40 mm. Hg.) occurred when the temperature of the externally applied water was nearest to that of the skin (30 to 35°C.). Landerer (working with Basler's ochrometer) found that the capillary pressure fell during a cold bath, while it was unchanged in a warm bath. Krauss (using his own apparatus, modified after Lombard) found that

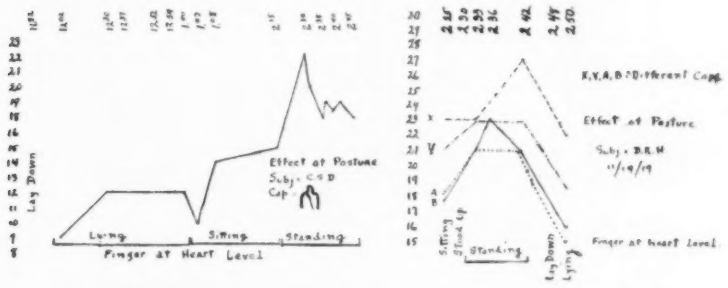

Fig. 6. Effect of posture on capillary blood pressure. Finger was at heart level throughout so that hydrostatic factor is excluded. The numbers at the left indicate pressure in millimeters of mercury.

the application of ice to his arm produced a fall of capillary pressure amounting to 20 mm. water and a rise of arterial pressure of 15 to 20 mm. Hg.

The effect of posture. The effect of posture on the capillary blood pressure is shown in figure 6. The hand was held at heart level in these observations in order to eliminate the hydrostatic effect. Hence our results represent the true postural effect. It will be seen that the lowest pressure occurs in the horizontal position, the highest in the vertical, and the capillary pressure in the sitting posture is midway between the two. Here again we see in the figure the individuality of some capillaries. There are two pairs of capillaries which differ from each other, yet the individual members of each couple act in close harmony as is evidenced by the parallelism of their pressure curves. Again the contractility of the capillaries suggests itself.

From this experiment it may be seen that capillary pressure determinations will vary according to the posture of the subject during the examination, although the hand be maintained at heart level. This has been impressed rather strongly on us very recently in connection with our clinical work on the bed cases in the hospital. In a number of these patients (in whom the capillary pressure was probably normal) readings ranging from 13 to 15 mm. Hg. were commonly obtained. These, of course, are a good deal lower than our original figures, which were made with the subject in the sitting position with the hand slightly below the heart level.

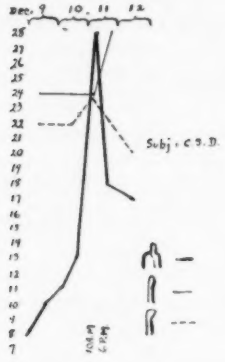

FIG. 7

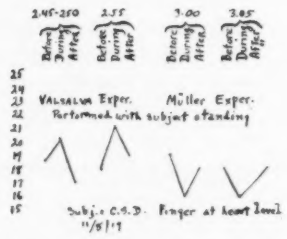

FIG. 8

Fig. 7. To show the variations in pressure in individual capillaries from day to day. While the pressure in an individual capillary may thus alter from day to day and from hour to hour, the average pressure in a group of capillaries is remarkably constant. See tables 2, 3 and 4.

Fig. 8. To show the effect of changes in intrathoracic pressure on the capillary pressure. Two observations on raising the intrathoracic pressure (left) and two on lowering the intrathoracic pressure (right) are given.

The following represents pressures in a series of capillaries studied on two consecutive days.

Diurnal variations. Hans Friedenthal says that capillary blood pressure varies by many hundreds per cent during the same 24 hours. In our experience this has never occurred. We have found on the contrary that the average pressure of a series of capillaries varies within relatively narrow limits from hour to hour, or even day to day. Some capillaries which we have followed in this manner have shown practically constant pressures, while others have shown very great variations.

This tendency for the pressure to vary in an individual capillary from day to day is illustrated in figure 7. The figure represents the pressures found in three different capillaries on four successive days. However much the pressure may fluctuate in an individual capillary there is nevertheless a remarkable uniformity in the results obtained when the pressures in a number of capillaries are averaged. This fact is well seen in the accompanying tables (tables 2, 3 and 4) dealing with the diurnal variations in capillary pressure. So far as our present observations go, therefore, there is practically no variation in the

TABLE 2

Determination of the pressure in a single capillary at different periods of the day

10:30 a.m.	15.00 mm. Hg. (Readings taken by Dr. H.)
1:00 p.m.	15.00 mm. Hg. (Readings taken by C. S. D.)
Lunch	
2:00 p.m.	14.16 mm. Hg. (Readings taken by C. S. D.)
2:45 p.m.	14.50 mm. Hg. (Readings taken by C. S. D.)
3:00 p.m.	15.17 mm. Hg. (Readings taken by C. S. D.)

TABLE 3

Determination of the pressure in four individual capillaries made on two days

DATE	TIME	CAPILLARY A	CAPILLARY B	CAPILLARY C	CAPILLARY D
December 6	10:30 a.m.	27	27	29	
December 7	9:15 a.m.	23	23	22	
	11:15 a.m.		24	23	22
	12:30 p.m.		25	24	22
	2:00 p.m.			26	23
	3:30 p.m.			24	24
	4:30 p.m.	24		24	24
	5:00 p.m.	24			22

average values for a given group of capillaries at different times of day and on different days.

The effect of intrathoracic pressure on capillary pressure. Disturbances of intrathoracic pressure affect both the arterial (37) and venous (38) blood pressure. We have performed one experiment to study its effect on the capillary pressure. Our results, given in figure 8, show that forced expiration with the glottis closed (Valsalva experiment) causes a rise, while forced inspiration with the glottis closed (Müller experiment) causes a fall in capillary pressure. The curves are practically mirror pictures of one another. Since a decreased intrathoracic

TABLE 4

Determination of the pressure in a group of capillaries studied on two consecutive days. Note the constancy of the pressure

DATE		Hg. PRESSURE	AVERAGE PRESSURE
		mm. Hg.	*mm.* Hg.
November 5		23.5	
		28.0	
		24.0	
a. m.	Ring finger	27.5	26½
		25.0	
		28.0	
		30.0	
		26.0	
		27.0	
	Small finger	25.0	24
		23.0	
		22.0	
		22.0	
		22.0	
		23.0	
		19.0	
		21.0	
		23.0	
		24.0	
		24.0	
p. m.	Middle finger	22.0	23
		22.0	
		22.0	
		22.0	
		23.0	
		25.0	
		26.0	
		27.0	
		23.0	
		25.0	
November 6		26.0	27
a. m.	Ring finger	28.0	
		30.0	
		30.0	
		23.0	
		24.0	
p. m.	Small finger	22.0	24
		27.0	
		23.0	

The pressure in the capillaries of the ring finger is practically constant for the two days; likewise the pressure in the small finger capillaries.

negative pressure lowers the arterial and raises the venous and capillary pressures, while an increased intrathoracic negative pressure produces the opposite circulatory conditions, it follows that capillary blood pressure is more closely dependent upon venous than it is upon arterial pressure.

von Basch (39), who reports results similar to the above, has also emphasized the association of capillary with venous pressure. With these observations in mind, a systematic study of capillary pressure in cases of cardiac insufficiency which determines a high venous pressure and in cases of emphysema, asthma, pleural effusions, etc., which determine disturbances of intrathoracic pressure, should prove fruitful.

The effect of venous compression on capillary pressure. A rubber band was applied around the little finger and the pressure in one and the same capillary was taken with varying degrees of compression. Very marked compression which closes both the arteries and veins reduces the capillary pressure to zero. Moderate compression, however, (which compresses the veins only) raises capillary pressure (18 mm. Hg.); when the rubber band is removed the pressure returns to normal.

Normal	15.0 mm. Hg.
Rubber band firmly applied	0
Rubber band loosely applied	18.0 mm. Hg.
Rubber band removed	15.5 mm. Hg.

Hence we see that increased venous pressure (produced by compression) increases the pressure in the capillaries. The effects of venous compression were previously studied by von Basch and later by Krauss. Both of these observers concluded that a high capillary pressure resulted from the compression of its veins. von Basch even went as far as to formulate an hypothesis which was to the effect that capillary pressure was an index of the amount of venous stasis.

SUMMARY

A method for the study of capillary blood pressure is presented which differs from all of the previous methods in the criterion at which the readings are taken. It depends on the production of stagnation of the flow of corpuscles in the capillaries.

With this method capillary pressure in man can be determined in the fingers and in the toes. For the study of capillary pressure in animals (cat, dog, rabbit) the shaved ear should be used. Our apparatus readily adapts itself for such study.

We have studied the capillary blood pressure in normal individuals (sitting posture). The average pressure was 22.2 mm. Hg.

The study on the effect of temperature showed that cold lowers and heat raises capillary blood pressure.

Posture was found to vary capillary pressure although the hand was at heart level at all times. It was lowest in the recumbent, highest in the standing and midway between in the sitting posture.

The diurnal variation, contrary to previous opinions, is very slight.

Increased intrathoracic pressure raises capillary pressure. Diminished intrathoracic pressure lowers capillary pressure.

Venous compression causes an increased capillary blood pressure.

We believe that the prevalent conception that the redness of the skin is due to its capillaries and that pallor means capillary emptying, is erroneous. While undoubtedly contributing somewhat to the color of the skin, nevertheless the rôle of the capillaries is rather slight. The venous plexuses principally contribute to the color of the skin. The collapse of these plexuses is the principal cause of skin pallor resulting from compression.

The simplicity and the accuracy of our method gives us hope that it may find a place in the laboratory and in the clinic.

We propose as the name of our apparatus, the "micro-capillary tonometer."[1]

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A PLETHYSMOGRAPHIC STUDY OF SHOCK AND STAMMERING IN A TREPHINED STAMMERER

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The experimental work here reported was done in January, 1920. The purpose of the experiment was to test the organic reactions accompanying stammering, with special reference to Dr. C. S. Bluemel's cerebral congestion theory of stammering, and to determine whether stammering, like shock, is accompanied by congestion in the brain, and hence by increased intracranial pressure, as was conjectured by me in the theoretical conclusions to "A plethysmographic study of shock and stammering" published in the April, 1919, number of this Journal.

Subject. The subject who took part in this experiment was born in Telsburg, Norway, November 14, 1875, and came to America in 1893. He went to school for nine years in Norway. He speaks Norwegian, Swedish and Danish as well as English, and used to speak Spanish; he stammers equally in all languages.

He is a laborer of low intelligence; he was a sailor for five years, a rigger for ten years, a pipe fitter for four years and a pile driver for two years. He attained a total score of 21, rating D, in Group Examination Alpha used in the United States Army, corresponding to a mental age of eleven years. His verbal imagery was the least vivid of any of the subjects tested in (5): his auditory was 1.03 compared with the average, 2.2: his kinesthetic was but 0.13 compared with the average, 0.8; and his visual but 0.27 compared with the average, for stammerers, 1.2. All types of his non-verbal imagery were vivid except the kinesthetic.

The subject spoke without hesitancy until, at the age of eight, he was so terrified on the ice by a boy who impersonated a bear that he was rendered speechless for two hours and has stammered ever since.

[1] The subject's salary and expenses were paid from a grant of $100 from the Committee on Grants of the American Association for the Advancement of Science.

His stammering was increased at the age of thirty when he was shot by a robber, the bullet splintering the skull bone over the right eye and necessitating the trephine described by Doctor Cobb. The skin of the noticeable dip or hollow covering the trephine was loose and free in its movements and the pulsations were often conspicuous.

As far as can be learned, the subject inherited no tendency to stammer. He sings normally and reads and speaks without hesitancy when alone. He frequently stammers on words beginning with H, L and R and sometimes repeats the first letters of certain words without contortions, but often avoids this stammering by using synonyms for the words he wishes to speak. He is not nervous or excitable, and is not apparently embarrassed or sensitive about his stammering. Pneumograms traced while the kymograph was running at high speed showed that he breathes correctly before he speaks, but continues to speak after his lungs are empty. In short, the physical aspect of his stammering is far more prominent than the mental aspect, and the impediment in his speech, which is of a common type, would not be difficult to correct in a younger man who was willing to work conscientiously.

The subject was examined at the Massachusetts General Hospital by Dr. Stanley Cobb on January 8, 1920. His report follows:

Complaint: Stammering.

Past History: Smallpox at 21. Gonorrhea at 23 and 24. At about this time he also had a hard chancre for which he was treated with pills and inunctions for three months. He does not know of any other sickness except that of August 30, 1905, when he received a gunshot wound in the right forehead which perforated the skull. He was taken to the Long Island College Hospital and operated on. Evidently the bullet was removed and a small trephine hole left open.

Physical Examination: Cranial nerves: Smell normal. Ocular movements are normal. No squint or difficulty in convergence. Muscles of mastication strong and equal. The sensation on the face is normal. There is no facial weakness. Hearing tests show slight deafness in the left ear. The Rinne test if referred to the left. The defect seems to be in the air conduction apparatus. The drum appears normal. Taste is normal. The pulse is regular and slow (see special report in experiments). No weakness of the sternomastoid or trapezius muscles. Tongue protrudes in midline. The fundi show normal vessels. In the right eye the disk is sharp but in the left the margins are slightly hazy. Visual acuity is within normal limits. He does not wear glasses.

Reflexes: Biceps, triceps, knee and ankle jerks are all equal and if anything slightly depressed. The superficial reflexes of the abdomen and scrotum are slightly more active than usual and equal on the two sides.

Motor system: The muscle groups are strong and symmetrical. There is

no disturbance of gait, no ataxia, asynergia or aphonia. There is a slight fine tremor of the extended fingers. Rhomberg test normal.

Sensory system: There is no disturbance of touch, pain or discrimination on any part of the body.

Endocrin system: Thyroid not enlarged. Bony development normal. The distribution of the hair on the body and face is normal. The testicles are in normal position and well developed.

Sympathetic system: The pupils react to light and accommodation. They are round and equal. Skin reactions are slight. There is no dermographia.

Skull: In the right fronto-temporal region 5 cm. from the midline is a defect in the bone irregularly circular in shape, its widest diameter being 2.5 cm. and its narrowest 2 cm. This pulsates visibly and when the patient leans over it is seen to bulge slightly.

Respiratory system: Lungs are clear. The nose, throat and tonsils are negative.

Cardiovascular system: The heart is not enlarged and the sounds are clear and without murmurs. The peripheral arteries are palpable but not thickened. The pulse is full. Blood pressure 110/80 (see special experiments). The aortic second sound is exaggerated and somewhat louder than the pulmonic second.

Alimentary system: The teeth are in fairly good condition except for three bad roots. The abdomen is level, soft and tympanitic. There are no masses felt.

Blood: No anemia. Wassermann reaction negative.

Mental status: General behavior: Normal, quiet and coöperative.

Stream of talk is slow but pertinent.

Mood: No depression or elation of spirits.

Special preoccupations: No worries, imaginations, delusions or hallucinations.

Orientation: Accurate for time, place and person.

Memory: Accurate for remote and recent events.

General Information: He knows the dates, names of the principal government officials, etc., but shows no interest in the affairs of the country.

Speech: There is a marked stammering, especially when embarrassed by the presence of a stranger. The sticking mainly occurs on the hard consonants. There is no aphasia, apraxia or astereognosis.

Diagnosis: An individual of the mentally dull type, probably not to be classed as a real defective, who has sustained a bullet wound of the right frontal region. There is no evidence of any brain injury.

Apparatus. The apparatus used in this experiment included the same Zimmerman kymograph, Sumner pneumograph, finger plethysmograph, piston recorder and the two electromagnets described in (6), pages 293 to 302, a less sensitive tambour than the one used in my earlier work, a second piston recorder like the first, and two forms of brain plethysmograph.

The first form of brain plethysmograph, which I will call the rubber plethysmograph, consisted of a hard rubber cup having an edge of soft rubber. A metal tube led out through the top side of the cup, and a rubber tube connected this with a syringe and piston recorder as in my earlier experiment. This plethysmograph was held in place by a single bandage passed around the subject's head from front to back. As the pressure of this bandage gave the subject a severe headache, most of the experiments were performed with the second form of brain plethysmograph, consisting of a glass funnel 4.7 cm. in inside diameter, which was cemented to the subject's forehead with collodion and removed with ether. This glass plethysmograph had two other advantages over the rubber one: it was not pressed against the forehead by bandages whose pressure varied with head movements, and an observer could detect sudden pulsations within the trephine which registered on the drum so much like movements, that in the records where the rubber plethysmograph was used I mistook them for movements and called in expert witnesses while the records were being traced to affirm that these abrupt rises were not due to movement in the large majority of cases.

With the apparatus described in (6), page 300, it was found that a rise of 1 mm. on the records always denoted an increase in volume of the brain of 3.0 cu. mm. when the rubber plethysmograph was used, and of 2.5 cu. mm. when the glass plethysmograph was used.

Procedure. The procedure and arrangement of apparatus was practically the same as described in (6), pages 302 to 306, except that the kymograph was run at higher speed to show the pulse. As five tracings were being made at the same time, it was very difficult to readjust one writing needle without throwing another out of adjustment. Being most interested in the brain tracing, I neglected the other writing needles when the one tracing the brain volume needed attention. For this reason, the needle which traced the finger volume and which moved freely only when it just touched the drum was frequently pressed too tightly against the drum to make a true tracing. The vasomotor reactions studied in (6), where this delicate pressure was kept constant, are far more reliable than those in this study.

As the pressure of the needle tracing the finger plethysmograms could not be kept uniform, no attempt was made to measure the finger plethysmograms; these were recorded simply as increases or decreases.

To determine what route the writing needle connected with the brain plethysmograph would have taken had the subject done no mental or

physical work and been given no stimulus, I placed the celluloid triangle along the crests of the highest pulses in the troughs of the Traube-Hering waves of the rest period immediately preceding and of that immediately following the period of work or disturbance. As the Traube-Hering waves were never large in these rest periods, and as the pulses at their troughs were much more uniform than those at their crests, this gave a fairly accurate reference line, though of course measurements made from it were necessarily approximate compared with those made from as accurate a reference line as was determined for the finger plethysmograms in (6). The lowest point on each plethysmogram, like the highest, was always considered to be the crest of a pulse at any phase of a Traube-Hering wave.

About half of the curves had to be discarded because of very slight leaks which developed in the brain plethysmograph when movements made by the subject loosened the bandage of the rubber plethysmograph or weakened some spot in the collodion which held on the glass plethysmograph; the discarded curves confirmed those retained as to rises and falls, but did not admit of accurate measurement. If the brain plethysmogram was reliable, the curve was retained, no matter how imperfect the finger plethysmogram.

The subject was seated in a large Morris chair so inclined that the trephine was nearly horizontal when he laid his head back in the chair; this put the least strain on the collodion which held on the glass plethysmograph.

An observer, seated close to the subject in most of these experiments, pressed a key whenever the subject moved his head, thereby enabling me to determine which abrupt rises in the brain plethysmograms were due to a rapid change in the brain's volume. In experiments arranged to study the effect of movements, it was found that small movements showed little if at all in the records, whereas big or quick movements were noticeable, raising the head or turning it to the right appearing to give a rise, lowering the head or turning it to the left appearing to give a fall. Curves containing head movements after which the writing needle did not return to normal were discarded.

Table 1 contains a brief summary of my results. The percentages of increases, decreases, no changes and complex reactions are given for both brain and finger volume: + denotes increase in volume; − denotes decrease in volume; 0 denotes no change in volume; +(−) denotes a prolonged increase followed by a short decrease; and so on. Rise in millimeters was measured to the highest point of the brain

plethysmogram from the path its writing needle would have traced had the subject's mind been a blank. This normal path was assumed to pass through the crest of the highest pulse at the trough of each Traube-Hering wave. The column entitled "Increase in height of brain pulse" gives the number of times greater in height the average pulse during the performance of an assigned task was than the normal pulse in the rest periods immediately preceding and immediately following the period of activity. The maximum pulse referred to in the next column is the highest brain pulse traced during the period of activity. t gives the time in seconds from the beginning of an assigned task to the time when the recording needle connected with the brain plethysmograph first attained its maximum rise. T gives the time in seconds from this maximum rise to the time when this recording needle first returned to normal, that is, to the path it would have taken had the subject's mind remained a blank. Or, if the time of maximum rise occurred while the subject was performing an assigned task, T was measured from the instant of completion of the task instead of from the end of t.

Every period of stammering while reading or speaking showed marked increase in brain volume usually accompanied by a greatly increased pulse for at least part of the period (see figs. 4, 5 and 6). Six periods of reading, averaging 77 seconds in length, gave rises of from 23 to 88 mm., averaging 49 mm. The maximum height of pulse registered during each reading period ranged from 1.7 to 5.0 times the normal size of pulse before and after the period of stammering, averaging 3.3 times the normal pulse. Twelve periods of talking, averaging 95 seconds in length, gave rises of from 32 to 70 mm., averaging 53 mm., and maximum pulse of from 1.4 to 7.0 times the size of the normal pulse, averaging 4.2 times the normal pulse. Thus there appeared to be slightly greater cerebral congestion during talking than during reading. The rise for these reading and talking periods, taken together, ranged from 23 to 88 mm., averaging 52 mm.; and the maximum pulse for the same periods ranged from 1.4 to 7.0 times the size of the normal pulse, averaging 3.9 times the normal pulse.

The maximum rise occurred on the average near the middle of the period in both reading and speaking. The brain volume and pulse became normal in from 20 to 46 seconds, averaging 33 seconds, after the end of the reading periods, and in from 0 to 82 seconds, averaging 37 seconds, after the end of the talking periods.

TABLE 1

Vasomotor changes accompanying various physical and mental states

WHAT SUBJECT WAS DOING	NUMBER OF CURVES	FINGER VOLUME	BRAIN VOLUME	BRAIN VOLUME RISE IN MILLIMETERS			INCREASE IN HEIGHT OF BRAIN PULSE			NUMBER OF TIMES MAXIMUM PULSE EXCEEDED NORMAL			*t*	*T*
				Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum		
		per cent	*per cent*											
Stammering	18	0 62 + 12 − 6 +− 19	+	23	52	88	Irregular			1.4	3.9	7.0	Middle	35
Reading aloud with no one in the room	1	+	+	53	53	53	1.8	1.8	1.8	2.5	2.5	2.5	Middle	18
Reading normally	6	0 67 + 33	+ 66 −+−17 0 17	−6	9	20	1.5	?	4.0	1.0	3.3	8.0	End	32
Silent reading	7	0 57 + 29 +− 14	+ 42 +−+29 0 29	0	14	29	1.0	1.0	1.0	1.2	1.4	2.0	Middle	55
Other mental work	8	+ 50 0 33 +− 17	+ 74 −+ 13 0 13	0	19	40	−?	+?	3.0	1.2	2.5	4.0	End	55
Physical work	6	+	+	19	21	25	2.4	5.4	8.0	2.8	7.5	12.0	Varied	54

Increased intrathoracic pressure	5	+ 20 0 40 − 40	+(−)	52	69	82	+	+	+	1.5	11.3	22.5	Start	36
Clearing throat	2		+(−)	63	66	70	+	+	+	1.6	1.7	1.8	Start	16
Sniffing	2	0	+	58	59	60	2.0	4.0	6.0	3.0	6.5	10.0	End	36
Deep breathing	5	0 67 − 33	+ 20 − 80	5	−12	−32	−1.7	−2.3	−3.0	1.0	3.3	10.0	End	43
Fear of stammering	4	0	+	15	28	45	+	+	4 0	2.1	4.5	5.8	?	?
Shock	10	0 87 − 13	+ 80 0 20	0	16	38	+	+	+	1.0	3.8	8.3	15	23

The finger plethysmograms gave about an equal number of vasodilatations and vasoconstrictions in both reading and speaking, two +, one −, one +−, one −+, one −+−, and ten no change; finger movements made it impossible to determine the other reactions.

It is important to determine what part of the increased intracranial pressure is due to stammering and what is due to the ordinary mental and physical work of reading or speaking. The subject read aloud in the room by himself without hesitancy; but there were the usual disturbances in breathing and the employment of superfluous effort which are usual in stammering whether reading alone or in public; and the single record (see fig. 3) in which the results were not injured by movement or by the needles' leaving the drum when no experimenter was in the room gave a maximum rise of 53 mm. in the middle of the 140 second reading period, an average pulse 1.8 times the normal pulse before and after reading, and a maximum pulse 2.5 times the normal, 25 mm. compared with 10 mm. The volume and pulse returned to normal more quickly, however, than after a period of severe stammering, this being accomplished in about 18 seconds. The finger plethysmograms showed marked vasodilatation. This single record indicates that a stammerer may have increased intracranial pressure while reading aloud or speaking whether he is stammering or not, but that this intracranial pressure is highest during periods of severe stammering.

A more satisfactory comparison would be that of the subject reading aloud a given passage and reading aloud another from the same book after he was cured. I was, fortunately, able to teach him in five days to read without hesitancy to any person who came into the room, and believe a comparison of records obtained while he was thus reading normally with the above stammering reading records will be a fair comparison of reactions to stammering and normal speech. He read aloud in this way in six periods free from movements in periods averaging 65 seconds in length (see fig. 7). Every time the subject breathed he raised his head, causing a distinct rise on the brain plethysmogram. A wave was thus caused in the curve which might be termed an indirect breathing curve. This curve was neglected in making measurements of changes in brain volume. One record gave no change in brain volume, one gave −6, + to 0, −6, and four gave rises from 12 to 20 mm., averaging 15 mm. The average pulse during these periods varied greatly and could be determined in only two records, in one increasing from 5 to 20 mm. and in the other decreasing from 12 to 8 mm. In two of the records the maximum pulse did not increase at all,

and in another it was as high as 8 times the normal, averaging 3.3 times the normal pulse. The greatest rise occurred in the middle of the period having the greatest average pulse and near the end of the other 5 periods. The time of recovery was 24 seconds in one, 40 seconds in another, and in doubt in 4. The finger plethysmograms showed a rise in two and no change in four of the records. There appears, therefore, to be much greater intracranial pressure in stammering reading than in normal reading, the average rise being 49 mm. compared with 15 mm.

To determine how much of the rise in normal reading was due to the mental work of the reading itself, I had the subject read silently seven passages in periods averaging 46 seconds (see fig. 2). The brain tracing showed no change in two, $+-+$ in two, and $+$ in three; where there was a $+$ in any part of a reaction, it ranged from 12 to 29 mm., averaging 20 mm., and will therefore account for the rise in normal reading aloud. The average pulse was about the same as the normal pulse, and the maximum pulse ranged from 1.2 to 2.0, averaging 1.4 times the normal. Hence the mental work of reading does not account for the increased pulse during normal reading; on the other hand, it will be seen that the physical work does account for this. The greatest rise occurred near the middle of the reading period and the time of recovery averaged 55 seconds, as it did in the case of other kinds of mental work. The finger plethysmograms showed one $+$, one $+-$, and four no changes.

Other kinds of mental work, including checking additions, multiplications and divisions (see fig. 2), and counting the number of E's on a page, gave reactions similar to silent reading in eight 1 to 3 minute periods. The brain volume remained the same in one, $0+$ in another, $(+)(-)+$ in another, $-+$ in another, and $+$ in four, the rise varying from 0 to 40 mm. and averaging 19 mm. The average pulse during periods of mental work was the same as the normal in four tracings, was lower in one, and was higher in three, as much as 3 times higher in one case; the maximum pulse was from 1.2 to 4.0, averaging 2.5 times the normal, being greater than that in reading silently. The maximum rise occurred near the end of the period of mental work, and the time of recovery ranged from 15 to 99 seconds, averaging 55 seconds. The finger plethysmograms showed no change in two curves, $+$ in three, $+-$ in one; and two were in doubt.

Periods of physical work on the ergograph used by Anderson and described in (1) page 42, gave six clear cut results (see fig. 1). There was

one fatigue period which lasted 184 seconds; the other test periods lasted one minute each. There was a rise of the brain plethysmogram with increased pulse in five periods, (+)(−)0 with larger pulse in the sixth. The rises ranged from 19 to 25 mm., averaging 21 mm., including the increased pulse which averaged 16 mm., about half of which is above the line drawn through the center of the pulse. The average pulse during physical work was from 2.4 to 8.0 times the normal, averaging 5.4 times the normal, and remained nearly uniform throughout the period of work, the maximum ranging from 1.2 to 1.7 and averaging 1.4 times the average pulse during the period of work. In the case of the long fatigue period the maximum rise occurred near the end; and in the short periods its position varied. The time of recovery ranged from 18 to 72 seconds, averaging 54 seconds. The finger plethysmograms gave + in five of the curves and (−)+ in the other: in this one the brief fall was due, no doubt, to the increased mental activity due to the change of task. Physical work, therefore, is accompanied by slight increase in both brain and finger volume, and by marked increase in brain pulse.

It seemed well, also, to study the brain volume during increased intrathoracic pressure, for this occurs with the muscular spasms which accompany stammering. The subject was asked, therefore, to clear his throat and also to bear down as if straining at the stool. In both cases the immediate rise was so great and so abrupt that I thought it was caused by a movement of the head until I saw through the glass plethysmograph that the skin over the trephine flattened out instantly at these times and that the subject did not move. Both showed a brief fall in brain volume after a marked rise, then a slow return to normal, the high pulse gradually decreasing (see fig. 9). The rise varied from 52 to 82 mm., averaging 68 mm. The maximum pulse averaged 1.7 times the normal in the clearing of the throat, and 11.5 times the normal in the bearing down, being 23 times the normal in one case, and even higher in discarded records where the piston of the recorder came out of its barrel. The time of recovery averaged 16 seconds for the clearing of the throat, and 36 seconds for the bearing down.

In one record chewing chocolate gave a rise in brain volume of 64 mm., the average pulse during chewing increasing from the normal of 9 to 20, and the maximum pulse to 45. This rise was not due to the pleasant sensation of the chocolate, as there was no appreciable rise during the half-minute he held this on his tongue before chewing; or

to the opening and closing of the mouth per se, as this gave a fall of 7 mm. and a low pulse when the subject opened his mouth every time he inhaled and closed it every time he exhaled. It must have been due to the physical work of chewing, and might be expected in any stammerer who forces words with his jaws.

A comparison of different kinds of breathing was made to learn what effect these had upon intracranial volume. Holding a deep breath caused a fall at first, then a rise until the subject breathed. Twelve seconds after the subject had kept his lungs empty for fourteen seconds, a maximum rise of 38 mm. occurred with maximum pulse nearly double the normal. Two records of sniffing (see fig. 8) gave rises of 58 and 60 mm. with average pulse during the period 6 times the normal in the first and twice the normal in the second. The time of recovery was 40 seconds in the first and 33 seconds in the second. There was no change in finger volume. The actions of clearing the throat and sniffing both tend to fix the diaphragm and thus cause increase of intrathoracic pressure.

Deep breathing, on the other hand, had quite a different effect (see fig. 8). Three periods gave (+)−, one +, and one − in the brain tracing, and one − and two no change in the finger tracing (two were obscured by movement). The single instance where the change in brain volume was + throughout was but 5 mm.; the four decreases ranged from 10 mm. to 32 mm., averaging 16 mm. The average pulse during this deep breathing either remained the same or decreased, the decrease ranging from 1.7 to 3 times as low as that of normal, averaging 1.8 times as low. The greatest decrease occurred at the end of each period.

In one record I had the subject read aloud normally, then breathe deeply, then read normally again, and then read silently, without pausing between these periods. The curve showed little change for these different periods of mental and physical work. The volume remained about 12 mm. above normal throughout the first normal reading period, averaged 10 mm. during the period of deep breathing, rose from 10 to 13 mm. during the second period of normal reading, and kept at 12 mm. during the period of silent reading, the pulse being higher for the silent reading period than for the other periods.

Fear of stammering was also compared with shock as in my earlier study, but in spite of my many efforts to get the subject to live over again an experience in which he feared that he would stammer, I was able to cultivate this emotion only four times. On the most successful

occasion (see fig. 5), I told him that a lady to whom my pupils found it most difficult to speak would enter the room in a few minutes and ask him some questions; she entered at the psychological moment. The four above mentioned brain plethysmograms gave rises of 15, 19, 33, and 45 mm. with increases in pulse, the average pulse increasing 4 times above the normal in the 33 mm. rise in figure 5. No change in finger volume could be detected in any of these curves.

My subject's reactions to shock confirmed those of Shepard, (7) and (8), discounting the fact that my subject was less emotional (see fig. 10). There was no change in brain volume in two of the ten reactions to the quick stimulus of a single loud noise, and an increase in the other eight ranging from 9 to 38 mm., averaging 20 mm. The maximum pulse ranged from no increase to an increase of 8.3 times the normal pulse, averaging 3.8 times the normal pulse. The maximum rise occurred from 5 to 18 seconds after the stimulus (30 seconds in the case of one stimulus whose length was in doubt), averaging 15 seconds. The time of recovery ranged from 14 to 34 seconds, averaging 23 seconds. The finger plethysmograms showed a decrease in one case, no change in six cases, and movements in the other three; this proves my assertion that this subject was far less emotional than the subjects in my earlier experiments (6).

Now and then I noted a decided rise in a rest period and asked my subject to tell me at the end of the experiment whether he was thinking of something pleasant or unpleasant just then; I found a reason for every such rise. In one case something exciting flashed in his mind for 4 seconds causing a rise of 12 mm. A very pleasant emotion for which he reported he was miles away caused a rise of 23 mm. (see fig. 1) and a maximum pulse 13 times the normal; the finger volume also increased in this case. I was unsuccessful in getting him to cultivate emotions at will, so had to study emotions by retrospective reports in this way.

In one case I had an assistant make a loud noise behind the subject in the middle of a speaking period and found that this made him stammer worse and greatly increased the brain volume; this noise startled the subject so that he moved enough to make a reference line inaccurate, but not enough to spoil the record.

So far as I know, there has been no previous work on a trephined stammerer. A comparison of my results with those of Berger, Mosso, Shepard and Weber upon normal speakers, summarized under "brain volume" at the bottom of table 1 (6, p. 289), shows that my results agree in every case with those of Mosso and Shepard, and agree with

those of Berger and Weber in the reactions to stimuli and to mental and physical work.

A few readings of the subjects systolic and diastolic blood pressure were taken at intervals while the experiments were in progress. The subject's normal blood pressure averaged 110/80. When asked a sudden question and put completely off his guard, Doctor Cobb found the subject's blood pressure to be 145/108. Bearing down sent the blood pressure up to 125/104, slight stammering to 122/?, physical work on the ergograph to 118/?. Increase in brain volume would seem, therefore, to have a high correlation with increase in blood pressure.

SUMMARY

1. Pronounced shock, fear of stammering and emotions of every kind always brought about increase in brain volume accompanied by increased size of pulse.

2. Mental work was accompanied by slight congestion in the brain in a majority of cases with little if any increase in pulse; physical work was accompanied by slightly more congestion in the brain with marked increase in size of pulse. Physical work was accompanied by slight vasodilatation in the finger; mental work, including silent reading, by vasodilatation in the finger in 38 per cent of the curves, by vasodilatation followed by vasoconstriction in 16 per cent, and by no change in 46 per cent.

3. Sniffing was accompanied by marked increase in brain volume with increased size of pulse; deep breathing by decrease with slightly decreased size of pulse.

4. Change of task was accompanied by increase in brain volume with increased pulse and was responsible for many temporary rises.

5. Normal reading aloud was accompanied by slightly less increase in brain volume than was silent reading.

6. Stammering was accompanied by much more marked increase in brain volume than could be accounted for by either the physical or mental work used in normal speech.

7. When the stammerer read as normal speakers do, there was a return of the brain volume to normal; it is reasonable to conclude that increase in brain volume is an important factor in the production of stammering.

8. In order to correct stammering, both the fear of stammering and the abnormal muscular contractions which usually accompany stammering must be eliminated.

BIBLIOGRAPHY

The following works have been specifically referred to in the text. For a complete bibliography, see (6), p. 323.

(1) ANDERSON: Circulatory reactions during physical and mental work, 1917. (This has not been published, but may be consulted in the Widener Library of Harvard University.)
(2) BERGER: Über die Korperlichen Ausserungen psychischer Zustande, Jena, 1904–7.
(3) BLUEMEL: Stammering and cognate defects of speech, New York, 1913.
(4) MOSSO: Über den Kreislauf d. Blutes im mench. Gehirn, 1881.
(5) ROBBINS: Psychol. Rev., 1920, xxvii, 38.
(6) ROBBINS: This Journal, 1919, xlviii, 285.
(7) SHEPARD: Amer. Journ. Psychol., 1906, xvii, 522.
(8) SHEPARD: The circulation and sleep, New York, 1914.
(9) WEBER: Der Einfluss psychischer Vorgange auf den Körper, Berlin, 1910.

EXPLANATION OF FIGURES

The top line in all figures is the time line, the second line is the stimulus line, the third line is the thoracic pneumogram, the fourth line is the finger plethysmogram and the bottom line is the brain plethysmogram.

Each notch in the time line represents two seconds unless otherwise stated.

A notch on the stimulus line indicates when a stimulus was given or when the subject began or stopped reading or speaking, and will be explained in the description of each record.

The top of the pneumogram indicates empty lungs and the bottom full lungs, just the reverse of the notation in my former monograph (6).

The top of each plethysmogram indicates vasodilatation, the bottom vasoconstriction.

The following curves are all typical reactions; I have avoided reproducing extremes or abnormal curves. If any reader wishes to see my other curves, he is invited to examine those in my album at the Bostom Stammerers' Institute.

Fig. 1 shows the changes in the brain pulse during a one-minute period of physical work on the ergograph. At the notch marked *2* the subject began to work and at *3* he stopped working. At *6* he was asked to remember what he was then thinking of; he reported at the end of the experiment that his mind was way off and that he was thinking of something very pleasant. Note that the height of the brain pulse increased during both the period of physical work and the pleasant emotion, and that the brain volume remained about the same. The finger volume increased in both cases, movement making the amount of the rise uncertain in the period of physical work. Three-fifths natural size.

Fig. 2 shows the changes in brain volume accompanying mental work. From notch *14* to notch *3* (the left hand *3*) the subject checked up a long division; from *3* to *16* he relaxed. At *15* my assistant accidentally dropped a small object. At *16* the subject picked up a book and read silently to *3* (the right hand *3*), where he again relaxed. At the notches marked *M* my assistant saw the subject move his head slightly. Note that the brain volume increased with little change in pulse during both periods of mental work; the brief initial rise in these two periods was probably caused by the marked changes in breathing recorded in the pneumogram. There was little if any change in finger volume. One-half natural size.

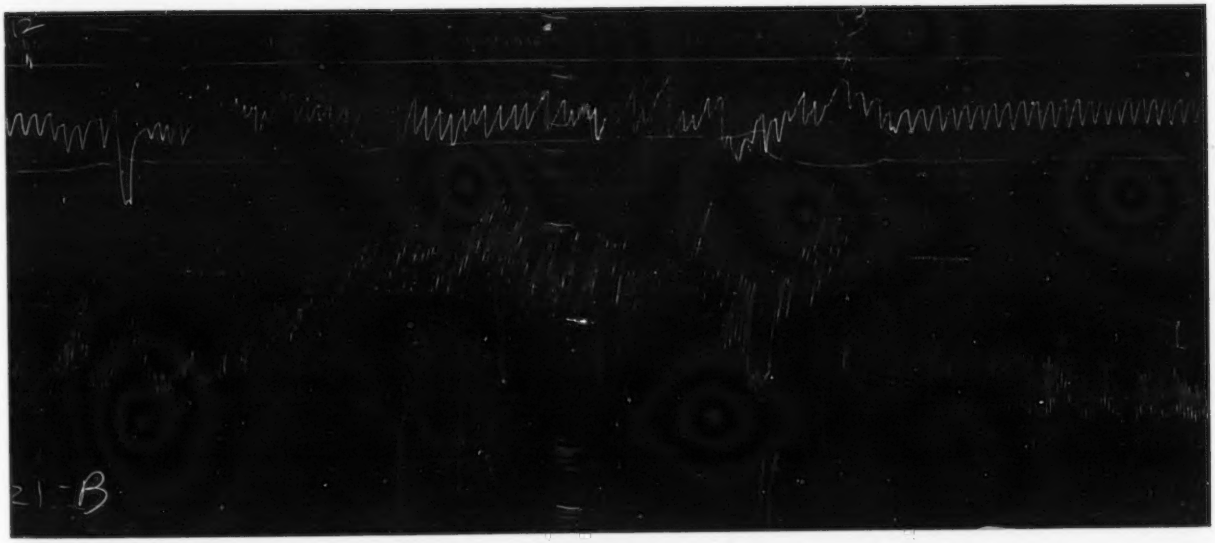

Fig. 3 shows the changes in brain volume and pulse while the subject read aloud in a room by himself without stammering before taking lessons. At *12* the experimenters left the room and the subject began to read; at *13* they returned and he stopped reading. Compare this figure with the next three and note that the brain volume increased as much during this period of reading in a room by himself as it did during some periods of stammering. One-half natural size.

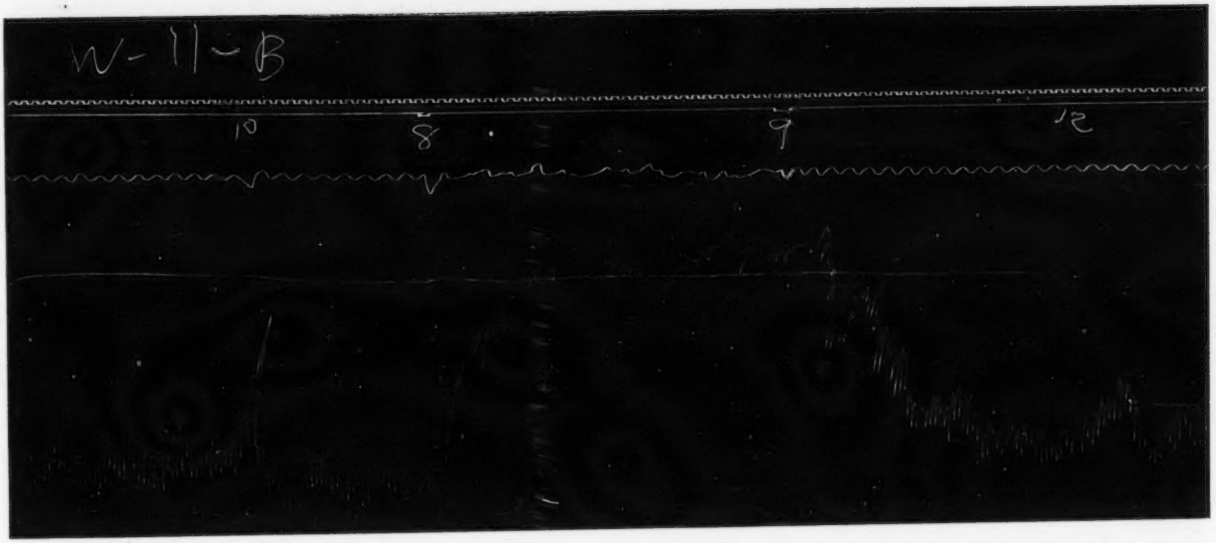

Fig. 4 shows the changes in brain volume during a long period of severe stammering while reading. At *8* the subject began to read aloud and at *9* he stopped reading. At *10* the subject yawned and at *12* I spoke to him. Note the marked rise in brain volume which accompanied stammering; the change in pressure of the writing needle on the drum occasioned by this big rise decreased the excursion of the needle on the drum and thus made a comparison of the pulse impossible. One-half natural size.

Fig. 5 shows the changes in brain volume and brain pulse accompanying fear of stammering. At *2* the subject was informed that a lady, to whom all stammerers found it very difficult to speak, would enter the room in about fifteen seconds and ask him some questions. She opened the door just before *3* and asked him brief questions from *3* to *4* which he answered briefly, stammering slightly. At *4* he relaxed. Note that both the brain volume and the height of brain pulse increased nearly as much during fear of stammering as during the actual stammering. One-third natural size.

Fig. 6 shows the changes in brain volume and brain pulse during stammering speech. At *8* the subject was told that a Harvard instructor would ask him some questions in about 15 seconds. At *5* this instructor asked the subject an unexpected question which the subject answered at *6*. The subject got much warmed up while answering question five and stammered more severely than usual in answer 6^5. At *7* the subject relaxed, and at *M* he moved his head slightly. Note the increase in both brain volume and pulse during the periods of stammering. One-half natural size.

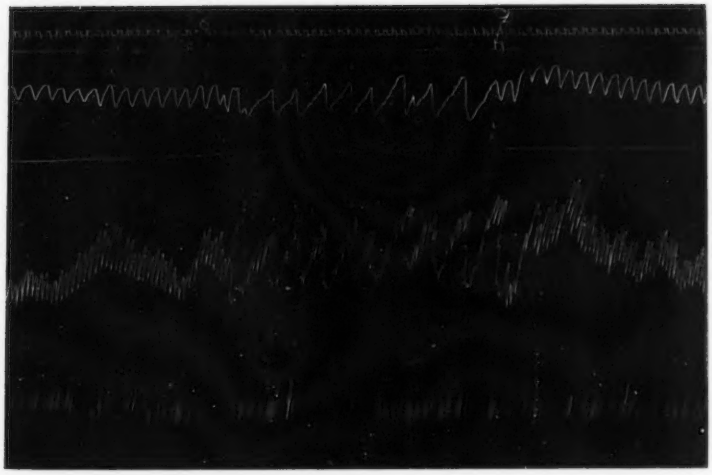

Fig. 7 contains a brain plethysmogram traced during a period of normal reading aloud after the subject had taken lessons. He read aloud normally from *8* to *9*, and read silently from *9* to the end of the curve. Note that there was little change in brain or finger volume, and that there was a noticeable secondary breathing wave in the brain plethysmogram. Two-fifths natural size.

Fig. 8 shows the comparative changes in brain volume and pulse during slow deep breathing and sniffing. The subject breathed slowly and deeply from *2* to *4*, relaxed from *4* to that point before *3* where the small waves in the large waves of the pneumogram show that he began to sniff. He sniffed as far as *3*, and then relaxed until the end of the curve. At *5* the kymograph stuck for a few seconds. Note that both brain volume and brain pulse decreased slightly during the period of deep breathing and increased considerably during the period of sniffing. Note the same secondary breathing wave in the brain plethysmogram that was seen in figure 7. One-half natural size.

Fig. 9 shows the changes in brain volume and pulse accompanying abdominal contractions. At *9* the subject was asked to bear down and at *12* he was asked to clear his throat. The length of time the subject held his breath represents the number of seconds he bore down; he did not wait for the signal at *9*. Note that the brain volume increased noticeably with both kinds of abdominal contractions. The initial abrupt rise under each *12* is not due to movement; one could see the skin over the trephine protrude every time the subject cleared his throat. Two-thirds natural size.

Fig. 10 shows the changes in brain volume accompanying shock. At *W* a shrill whistle was blown unexpectedly. The clock stopped running for a few minutes at the beginning of this curve. Note that the brain volume increased soon after the whistle was blown and that the finger volume remained constant. Two-fifths natural size.

THE CHEMICAL CONSTITUTION OF ADENINE NUCLEOTIDE AND OF YEAST NUCLEIC ACID

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I. A COMPARISON OF THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM YEAST NUCLEIC ACID WITH THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM THE INDIVIDUAL NUCLEOTIDES

After an extended study of the rate at which phosphoric acid is split from yeast nucleic acid by hydrolysis with mineral acid, Jones and Riley[1] concluded that the nucleic acid liberates its phosphoric acid by two widely different laws: and they predicted that if the individual nucleotides were ever prepared from yeast nucleic acid and examined in this respect, each of the purine nucleotides would be found to obey one of these laws and each of the pyrimidine nucleotides would obey the other. Various preparations of mixed nucleotides were afterwards obtained and their conduct justified this assumption since each nucleotide preparation was found to set free its phosphoric acid in accordance with the proportion of purine and pyrimidine material that it contained.[2] Finally Jones and Kennedy[3] found that the nucleotide groups are burned from yeast nucleic acid by potassium permanganate in a definite order, adenine nucleotide being the last to go; so that it was possible to obtain pure adenine nucleotide in this way.

The substance consists of characteristic long transparent needles of the composition $C_{10}H_{14}N_5PO_7.H_2O$ and its possession furnished an excellent opportunity to ascertain the rate at which phosphoric acid is set free from a purine nucleotide. This rate was found to be the same as that of the previously determined rate for guanine nucleotide,[4]

[1] Journ. Biol. Chem., 1916, xxiv, i.

[2] Jones and Germann: Journ. Biol. Chem., 1916, xxv, 100. For the analytical data see Jones and Read, Journ. Biol. Chem., 1917, xxix, 123.

[3] Journ. Pharm., 1918, xii, 253; 1919, xiii, 45.

[4] Jones and Read: Journ. Biol. Chem., 1917, xxxi, 337.

both being the rate that Jones and Riley[5] had predicted for purine nucleotides.

This relation is expressed diagrammatically in figures 1 and 2, which are constructed with the same system of coördination and the same linear units. Abscissae represent time and ordinates represent weights of

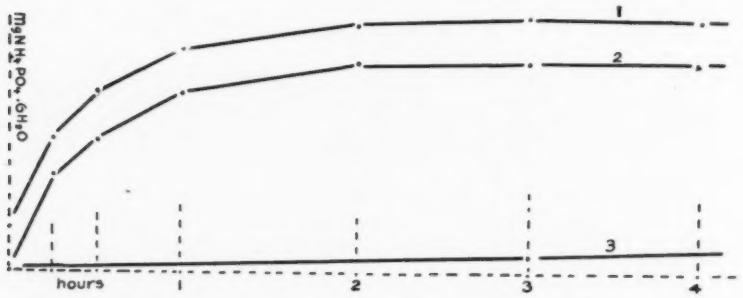


Fig. 1. The two curves at the top show the rate at which phosphoric acid is set free from the purine nucleotides. The straight line shows the rate for the pyrimidine nucleotides.

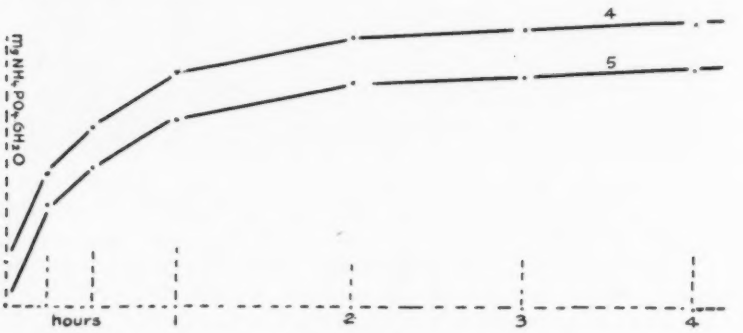


Fig. 2. The upper curve is a fusion of *3* with *1* or *2*. The lower curve shows the rate at which phosphoric acid is set free from yeast nucleic acid.

phosphoric acid (expressed in terms of ammonium magnesium phosphate). In order to avoid confusion, the curves in each diagram have different origins placed vertically above one another.[6]

[5] Journ. Biol. Chem., 1916, xxiv, i.

[6] For analytical data see end of the article.

The upper curve of figure 1 was constructed from experimental data obtained with guanine nucleotide three years ago by Jones and Read.[7]

The lower curve of figure 1 was similarly constructed from experimental data obtained more recently by Jones and Kennedy[8] with adenine nucleotide.

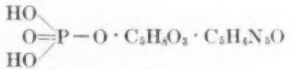
The two curves are practically identical and may be called the law for purine nucleotides.

The straight line of figure 1 was drawn from experimental data obtained by Jones and Read[9] with a mixture of the two pyrimidine nucleotides. It shows the very slow regular rate which may be called the law for pyrimidine nucleotides.

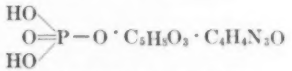
The upper curve of figure 2 was constructed by fusing the pyrimidine curve with either one of the purine curves of figure 1.

The lower curve of figure 2 was constructed from experimental data obtained with yeast nucleic acid, and it practically coincides with the lower curve.

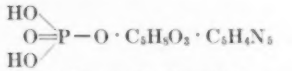
This coincidence points to a very definite conclusion. If it be granted that the rates are expressions of phosphoric acid linkages, then the phosphoric acid linkage of yeast nucleic acid must coincide with the phosphoric acid linkages of its four component nucleotides.



Guanine Nucleotide[10]



Cytosine Nucleotide[11]



Adenine Nucleotide[12]

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[7] Journ. Biol. Chem., 1917, xxxi, 337.

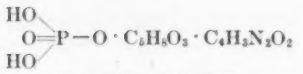
[8] Journ. Pharm., 1918, xii, 253; 1919, xiii, 45.

[9] Journ. Biol. Chem., 1917, xxxi, 39.

[10] Jones and Richards: Journ. Biol. Chem., 1914, xvii, 71.

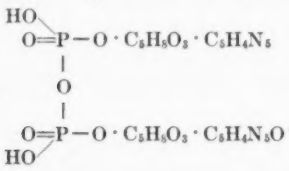
[11] Thannhauser and Dorfmüller: Ber. d. d. chem. Gesellsch., 1918, li, 467. Zeitschr. f. physiol. Chem., 1919, ciiii, 65.

[12] Jones and Kennedy: Journ. Pharm., 1918, xii, 253; xiii, 45.

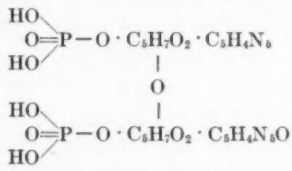


Uracil Nucleotide[13]

If yeast nucleic acid is a chemical combination of the four nucleotides, then in this union the phosphoric acid groups of the nucleotides must not be disturbed. No additional phosphoric acid linkages can be introduced. The four nucleotides possess together eight replaceable hydrogen atoms; so also, yeast nucleic acid must contain eight replaceable hydrogen atoms. Therefore the nucleotide linkages of yeast nucleic acid cannot be through the phosphoric acid groups: the linkages cannot involve any one of the four phosphoric acid groups.[14]



Two nucleotides united through their phosphoric acid groups



Two nucleotides united but not through their phosphoric acid groups

Until very recently it was conceded by everyone that if the nucleotide linkage of yeast nucleic acid is not through its phosphoric acid groups then it is naturally through the carbohydrate groups. That question is taken up in the following section.

II. A COMPARISON OF THE RATE AT WHICH THE PURINES ARE SET FREE FROM YEAST NUCLEIC ACID WITH THE RATE AT WHICH THE PURINES ARE SET FREE FROM THE INDIVIDUAL PURINE NUCLEOTIDES

At the time the phosphoric acid studies were made with guanine nucleotide and adenine nucleotide, the rates at which they set free their guanine and adenine were ascertained. It is exceedingly rapid and the same for both purines and for yeast nucleic acid. The rapidity

[13] Levene: Journ. Biol. Chem., 1920, xli, 1.

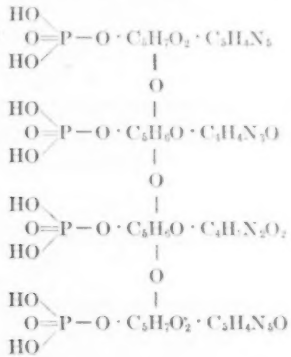
[14] The argument here used is essentially that which was employed for the same purpose by Jones and Read (Journ. Biol. Chem., 1917, xxix, 123). Their argument is today as sound as it was when they wrote.

is such that the time required to dissolve the nucleotide in the hydrolytic agent has to be considered and in the case of yeast nucleic acid the other products of hydrolysis make the determination of the liberated purines very difficult. But one can conclude from the data without hesitation that the purines are set free from nucleic acid and from the individual purine nucleotides with the same rapidity.

Again, the exceeding slowness with which the pyrimidines are split by hydrolysis from yeast nucleic acid makes quantitative work impossible. But the same slowness characterizes the pyrimidine nucleotides.

Therefore the argument which was used above to show that the nucleotide linkages of yeast nucleic acid do not involve the phosphoric acid groups, may now be used to show that the nucleotide linkages do not involve the purine groups nor probably the pyrimidine groups.

This leaves the carbohydrate groups and indicates the formula:



III. A COMPARISON OF THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM ADENINE NUCLEOTIDE WITH THE RATE AT WHICH ADENINE IS SET FREE FROM ADENINE NUCLEOTIDE

The longest known nucleotide (though not a nucleotide of yeast nucleic acid) is the substance that Liebig[15] discovered in meat extract and called inosinic acid. It was afterwards shown contemporaneously by Bauer[16] and by Neuberg and Brahn[17] that inosinic acid is com-

[15] Liebig's Annalen, 1847, lxii, 317.

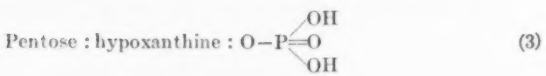
[16] Hofmeister's Beitr., 1907, x, 345.

[17] Biochem. Zeitschr., 1907, v, 439; Ber. d. d. chem. Gesellsch., 1908, xli, 3376.

posed of the groups of three substances, viz., phosphoric acid, pentose and hypoxanthine. Hence inosinic acid may have any one of three structures, i.e., any one of the three groups may be the central group connecting the other two.

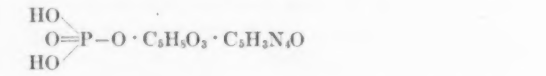
(1)

(2)

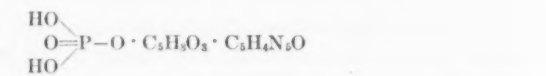


(3)

Liebig knew that inosinic acid is a dibasic acid. This excludes formula (2). Haiser[18] found that by acid hydrolysis inosinic acid loses its hypoxanthine much more rapidly than its pentose.[19] This excludes formula (3) and leaves the correct formula (1):



This method may be used to find the gross structure of any purine nucleotide (but obviously not of a pyrimidine nucleotide) and was applied to guanine nucleotide by Jones and Read[20] who found that the substance is a dibasic acid that forms a dibrucine salt and that, by acid hydrolysis, the nucleotide loses its guanine very much more rapidly than its phosphoric acid. These two facts necessitate the following arrangement of the three groups:



[18] Monatshefte f. Chem., 1895, xvi, 190.

[19] Haiser mistook the pentose for trioxyvaleric acid. Both substances have the formula $C_5H_{10}O_5$.

[20] Journ. Biol. Chem., 1917, xxxi, 337.

The curves of figure 3 are constructed from experimental data obtained with adenine nucleotide. The upper curve expresses the rate for adenine and the lower curve the rate for phosphoric acid. In five minutes three times as much adenine is set free as phosphoric acid: or, the liberation of adenine is nearly complete in thirty minutes, that of phosphoric acid only after two hours.

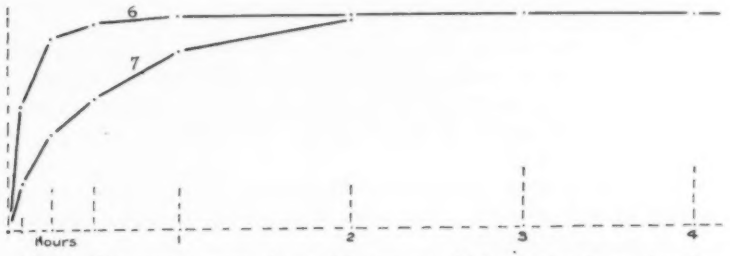
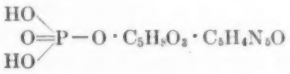


Fig. 3. The upper curve shows the rate at which adenine is set free from adenine nucleotide. The lower curve shows the slower rate for phosphoric acid.

Adenine nucleotide forms a dibrucine salt and conducts itself toward alkalis like a dibasic acid. Hence its groups must be arranged as indicated in the formula



IV. THE ACIDITY OF ADENINE NUCLEOTIDE

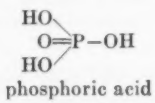
In the article that follows it will be assumed that the nucleotides conduct themselves like acids toward indicators and alkalis. One cannot work long with the nucleotides without knowing that this is true of them all. When a lead salt is decomposed with sulphuretted hydrogen the filtrate from the lead sulphide is strongly acid to litmus. As the matter is of considerable importance, however, an exact examination of adenine nucleotide was made.

A weighed portion of the nucleotide (50 mgm.) was covered with water and titrated with 0.104 N sodium hydroxide using phenolphthalein for the indicator; 2.69 cc. were required. A second portion of 50 mgm. was titrated using methyl orange for the indicator. Half as much alkali was required.

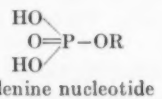
STANDARD ALKALI USED FOR 50 MGM. OF ADENINE NUCLEOTIDE		THEORETICALLY REQUIRED FOR	
Using phenolphthalein	Using methyl orange	Two equivalents of hydrogen	One equivalent of hydrogen
cc.	*cc.*	*cc.*	*cc.*
2.69	1.35	2.68	1.31

When free phosphoric acid is titrated with sodium hydroxide using methyl orange as an indicator exactly one equivalent of hydrogen is neutralized; but if phenolphthalien is used as an indicator, twice as much alkali is required, which of course corresponds exactly to two equivalents of hydrogen.

Adenine nucleotide therefore conducts itself toward alkalis exactly like free phosphoric acid. This is what one would expect a substance of its structure to do.



phosphoric acid



adenine nucleotide

Experimental

Weighed portions of adenine nucleotide, placed in flasks provided with condensing tubes and treated with twenty parts of 5 per cent sulfuric acid, were heated in a boiling water bath. At various intervals from 15 minutes to 3 hours, a flask was removed from the water bath and both free phosphoric acid and free adenine were quantitatively determined as follows. The hot fluid was made alkaline with ammonia and treated with a slight excess of magnesia mixture. After standing 5 hours, the crystalline magnesium ammonium phosphate was filtered off, allowed to dry in the air and weighed. The weight was divided by the weight of the nucleotide used in the experiment to obtain the weight per gram of nucleotide, so that the results of various experiments could be directly compared with one another. It was also found convenient not to calculate the corresponding amount of phosphorus but to express the phosphoric acid throughout in terms of magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$).

The ammoniacal filtrate containing adenine was treated with a solution of silver nitrate in ammonia and the precipitated adenine-silver

compound was filtered off, thoroughly washed, suspended in water and decomposed with hydrochloric acid. After filtration from silver chloride, the acid fluid was evaporated to dryness (with the usual precautions) for the expulsion of all but a trace of free hydrochloric acid, and a solution of the residue in a little warm water was treated with a slight excess of picric acid. The precipitated crystalline adenine picrate was filtered off, allowed to dry in the air and weighed. This weight was divided by the weight of the nucleotide used in the experiment and the corresponding amount of adenine was calculated.

In addition, the total amount of phosphoric acid obtainable from the nucleotide after completely burning was determined and, for comparison, was expressed in terms of magnesium ammonium phosphate per gram of nucleotide.

The results are given in table 1.

Results obtained in a similar way by Jones and Read with guanine nucleotide are given in table 2, and for comparison the results obtained with yeast nucleic acid by Jones and Riley are given in table 3.[21]

TABLE 1

Adenine nucleotide

NUCLEOTIDE USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 \cdot 6H_2O$			ADENINE PICRATE		CALCULATED ADENINE	PER CENT OF THEORETICAL (0.3700)
		Obtained	Per gm. of nucleotide	Per cent of theoretical (0.671)	Obtained	Per gm. of nucleotide		
0.2161	5 min.	0.0304	0.1409	21.0	0.1253	0.5800	0.2146	58.0
0.6204	15 min.	0.1915	0.3087	46.0	0.5569	0.8976	0.3330	90.0
0.6167	30 min.	0.2566	0.4160	62.0	0.5980	0.9696	0.3587	97.2
0.6302	1 hr.	0.3552	0.5637	84.0	0.6285	0.9973	0.3700	100.0
0.6111	2 hrs.	0.4084	0.6683	99.5	0.6017	0.9844	0.3652	98.7
0.6123	3 hrs.	0.4088	0.6678	99.7	0.6154	1.0051	0.3729	100.8
0.3033	4 hrs.	0.2023	0.6669	99.4	0.1114	0.9930	0.3674	99.3
0.3247	Total	0.2159	0.6646	99.5				
0.3103	Total	0.2060	0.6639	98.9				
	Theoretical		0.6710	100.0		1.000	0.370	100.0

[21] Two values are the results of later experiments.

TABLE 2

Guanine nucleotide[22]

NUCLEOTIDE USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 . 6H_2O$			GUANINE		
		Obtained	Per gm. of nucleotide	Per cent of total	Obtained	Per gm. of nucleotide	Per cent of total
0.4234	5 min.	0.0499	0.1178	18.8	0.0835	0.1972	50.0
0.5030	15 min.	0.1348	0.2680	42.8	0.1747	0.3473	88.1
0.5000	30 min.	0.2004	0.4008	64.0	0.1901	0.3802	96.3
0.3487	1 hr.	0.1881	0.5395	86.2	0.1396	0.4002	101.5
0.3360	2 hrs.	0.2017	0.6000	95.8	0.1310	0.3899	98.9
0.3448	3 hrs.	0.2158	0.6259	100.0	0.1360	0.3944	100.0
0.4112	4 hrs.	0.2539	0.6174	98.6	0.1617	0.3932	99.8

TABLE 3

Yeast nucleic acid[23]

NUCLEIC ACID USED	TIME	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 . 6H_2O$						
		Obtained	Per gram	From pyrimidine nucleotides	I Per cent of half the total (0.295)	From purine nucleotides	II Per cent of half the total	Sum of I and II
1.0031	15 min.	0.1374	0.137	0.003	0.85	0.134	40.4	41.25
1.0001	30 min.	0.2090	0.209	0.005	1.70	0.204	61.3	63.0
0.8642	1 hr.	0.2315	0.269	0.010	3.40	0.259	87.8	91.2
0.9837	2 hrs.	0.3125	0.318	0.020	6.80	0.298	101.0	107.8
0.9251	3 hrs.	0.3038	0.329	0.030	10.20	0.299	101.0	111.2
1.0305	4 hrs.	0.3488	0.338	0.040	13.60	0.298	101.0	114.6
0.8333	5 hrs.	0.2876	0.345	0.050	17.00	0.295	100.0	117.0
0.9927	6 hrs.	0.3565	0.359	0.060	20.40	0.299	101.0	121.4
0.8179	Total	0.5112	0.590	half the total = 0.295				

TABLE 4

Pyrimidine nucleotides[24]

NUCLEOTIDES USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 . 6H_2O$		
		Obtained	Per gm. of nucleotides	Per cent of total
1.0362	3 hours	0.0647	0.0624	10.0
0.3587	Total	0.220	0.624	

[22] Table of Jones and Read. Journ. Biol. Chem., 1917, xxxi, 337. The four-hour period is added.

[23] Data taken from article of Jones and Riley, Journ. Biol. Chem., 1916, xxiv, i, except 15-minute and 30-minute periods.

[24] From data of Jones and Read, Journ. Biol. Chem., 1917, xxxi, 39.

THE ACTION OF BOILED PANCREAS EXTRACT ON YEAST NUCLEIC ACID

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The earliest investigations of nucleic acid had shown that by mild acid hydrolysis, the purines are set free with part of the phosphoric acid and part of the carbohydrate; but that violent hydrolytic processes are required to set free the pyrimidines with the remainder of the phosphoric acid and carbohydrate. A great amount of ingenuity was therefore not required to formulate a hypothetical structure for nucleic acid. The substance must be composed of four complexes, all of which contain a group of phosphoric acid and a group of carbohydrate but each complex contains a different one of the four nitrogenous groups.

Change the word "complex" to the word "nucleotide" and the above becomes essentially the modern nucleotide theory of the constitution of yeast nucleic acid.

But one important matter concerning the constitution of yeast nucleic acid was not touched upon by the early investigators. At what points are the nucleotides joined together to form yeast nucleic acid or, in other words, what is the mode of nucleotide linkage in yeast nucleic acid? Without any experimental evidence it was finally agreed that the nucleotide linkage is through the phosphoric acid groups and this assumption remained undisturbed until Jones, Germann and Read furnished the experimental evidence to show that this mode of nucleotide linkage is not correct. The principal object of the present paper is to describe an experiment which proves this in a very simple and striking way.

Pig's pancreas contains a variety of active agents (ferments) which decompose nucleic and further act on its decomposition products. When an aqueous extract of pancreas is boiled, all of these active agents are destroyed but one, viz., the one that decomposes yeast nucleic acid into its nucleotides.

An aqueous extract of pig's pancreas was boiled and filtered. Yeast nucleic acid was added to the clear extract and the mixture was allowed to digest for 20 hours at 40°, when the nucleic acid had disappeared[1] and a mixture of the four nucleotides could be isolated.

This ferment (if it is a ferment) acts rather rapidly at first but more slowly afterwards. It is exceedingly more active at 40° than at 20° and exhibits about the same activity whether the solution be amphoteric, faintly alkaline or acid to litmus. It is not present in the liver nor the spleen and does not decompose thymus nucleic acid.[2] In the decomposition of yeast nucleic acid by this ferment neither phosphoric acid nor purine bases are set free and deaminization does not occur. *But when nucleic acid is converted into nucleotides by this active agent there is not the slightest change in the acidity of the solution.*

Yeast nucleic acid is represented below by two formulas which differ from one another in only one respect. Formula I has its nucleotide linkages through the phosphoric acid groups; formula II has its nucleotide linkages through the carbohydrate groups.

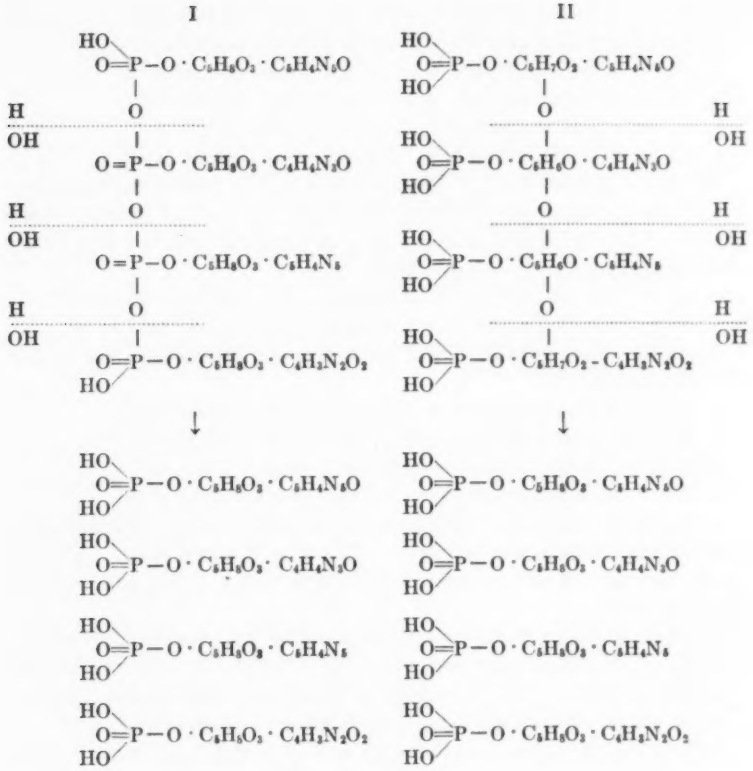
If formula I is correct, the conversion of nucleic acid into its nucleotides should be attended by a marked increase in acidity: but if formula II is correct, there should be no increase in acidity. If formula I correctly represents the structure of nucleic acid, then the increased acidity due to the decomposition of 2 grams of nucleic acid into its nucleotides should require about 8 cc. of 0.1 N sodium hydroxide for neutralization. But as a matter of fact it was not possible to demonstrate any change in acidity with sensitive indicators when 2 grams of nucleic acid was decomposed into its nucleotides.

This is a crucial experiment that decides against the phosphoric acid linkage and, I think, in favor of the carbohydrate linkage since the other possibilities are only of academic interest.

I cannot feel responsible for, nor even interested in the impossibility of two carbohydrate groups uniting with one another, and presume that such a union will be conceded without any proof if the other conceivable possibilities are excluded.

[1] The merest trace of nucleic acid can be detected in such a solution by the addition of sulphuric or hydrochloric acid.

[2] This is curious. An active agent is present in animal pancreas which is specifically adapted to plant nucleic acid. It suggests evolutionary matters.



EXPERIMENTAL

A mixture of 2 kilos of carefully trimmed and ground pig's pancreas, 2 liters of water and 30 cc. of chloroform was allowed to digest for 12 hours at the room temperature in a tightly closed vessel with frequent and violent agitation. After the tissue had by this means become thoroughly penetrated with chloroform, the mixture was placed in a thermostat and allowed to digest at 40° for 2 days, when it was cooled and filtered. The clear, pale yellow filtrate was then boiled, filtered from a small coagulum and after cooling was preserved with chloroform for use in the following experiments. The data given are selected from a large amount of similar data.

a. 50 cc. of boiled extract + 0.250 gm. yeast nucleic acid[3]

[3] In all experiments the digesting material was preserved with chloroform.

b. 50 cc. of boiled extract + 0.375 gm. yeast nucleic acid
c. 50 cc. of boiled extract + 0.500 gm. yeast nucleic acid
d. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid
e. 50 cc. of boiled extract + 1.000 gm. yeast nucleic acid
f. 50 cc. of boiled extract + 1.250 gm. yeast nucleic acid
g. 50 cc. of boiled extract + 1.500 gm. yeast nucleic acid

All were digested at 40°. After 24 hours *a*, *b*, *c*, *d* and *e* gave no cloud with H_2SO_4; *a* and *b* gave nothing with HCl, but *c*, *d* and *e* gave a slight cloud. After 48 hours *a*, *b* and *c* gave nothing with HCl; *d* and *e* only an opalescence; *f* and *g* a faint cloud.

a. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid at 20°
b. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid at 40°

After 12 hours sulphuric acid gave a dense precipitate with *a* but nothing with *b*.

a. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
b. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
c. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
d. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid

a was made amphoteric to litmus; *b* was made faintly alkaline; *c* was markedly alkaline; *d* was left acid. All behaved about alike. After 20 hours digestion at 40° all gave a faint cloud with H_2SO_4 but nothing after 36 hours.

a. 50 cc. of boiled pancreas extract + 0.750 gm. yeast nucleic acid
b. 50 cc. of boiled pancreas extract + 0.250 gm. thymus nucleic acid
c. 50 cc. of boiled spleen extract + 0.250 gm. yeast nucleic acid
d. 50 cc. of boiled liver extract + 0.250 gm. yeast nucleic acid
e. 50 cc. of phosphate mixture (pH = 6.4) + 0.250 gm. yeast nucleic acid.

Digested at 40°. After 19 hours *a* failed to give a cloud with H_2SO_4 but *b*, *c*, *d* and *e* all gave dense precipitates with H_2SO_4 even after 48 hours.

Three cubic centimeters of boiled pancreas extract were diluted with 10 cc. of water and treated with 4 drops of a solution of brom-cresol purple. On comparison of the color with a set of standard colors its acidity (pH) was found to be between 6.0 and 6.4 (about 6.2). A larger quantity of the extract was then titrated with 0.1 N sodium hydroxide to an acidity (pH) of 6.4. Changes in the acidity of such a solution can easily be detected with brom-cresol purple.

One and one-half gram of yeast nucleic acid were dissolved in 75 cc. of the above extract and the solution was titrated with 0.1 N sodium hydroxide to an acidity of 6.4, i.e., until the color which it gave with 4 drops of brom-cresol purple exactly matched the color similarly given by the extract. The extract and the solution were then digested at

40° for 48 hours. The nucleic acid had entirely disappeared and the color produced by brom-cresol purple with the solution exactly matched the color produced with the extract. The acidity of both solution and extract had not changed from 6.4. A trace of 0.1 N hydrochloric acid caused a very perceptible change in the acidity of the solution.

This experiment confirms many less accurate ones that were made. In the tests for the influence of acidity on the activity of the ferment no faintly alkaline solution ever became amphoteric to litmus and no amphoteric solution ever became acid. In fact, no change at all in the tint given to litmus was ever noticed as digestion proceeded.

50 cc. boiled pancreas extract

50 cc. boiled pancreas extract + 0.750 gm. yeast nucleic acid

The two solutions were digested at 40° for 48 hours, when the nucleic acid had entirely disappeared.

Each solution was made alkaline with ammonia and treated at the boiling point with an excess of magnesia mixture, when perfectly white crystalline ammonium magnesium phosphate was precipitated. This was filtered the next day, allowed to dry and weighed.

$MgNH_4PO_4.6H_2O$ from the experiment 0.5474

$MgNH_4PO_4.6H_2O$ from the blank 0.5469

An experiment was made with boiled extract which involved 125 gm. of yeast nucleic acid. After the nucleic acid had disappeared by digestion at 40° the product was heated to boiling and treated with neutral lead acetate as long as the reagent gave a precipitate in the hot fluid. This precipitate which consists principally of lead phosphate was filtered off with a pump and the pale yellow filtrate was treated in the warm with more lead acetate. At first no precipitate was produced but after the addition of a sufficient excess of the reagent a copious granular precipitate was thrown down. After cooling, the precipitated lead salts of the nucleotides were filtered off, washed, suspended in warm water and decomposed with sulphuretted hydrogen. The filtrate from lead sulphide was evaporated at 45° under diminished pressure and the nucleotides were thrown out and dried with absolute alcohol. This product is undoubtedly identical with the mixture of nucleotides formerly obtained by Jones and Richards[4] from yeast nucleic acid by treatment with an extract of pig's pancreas that had previously been digested for a long time at 40°. It contains all four nucleotides and forms a mixture of crystalline brucine salts. As the separation of the nucleotides from one another is foreign to the principal point of this paper, the matter will be taken up later in a separate article.

[4] Journ. Biol. Chem., 1915, xx, 25.